DouxMatok

incredo SUGAR

25 February 2021

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: GRAS Notice for Synthetic Amorphous Silica as a Carrier in White Sugar

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, DouxMatok Inc., as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that synthetic amorphous silica (SAS) is GRAS on the basis of scientific procedures for as a carrier in white sugar; the food use of SAS, is therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for DouxMatok's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

David Tsivion
VP R&D
DouxMatok Ltd.

Email: david.tsivion@douxmatok.com
Tel: +972-3-8060200

GRAS NOTICE FOR SYNTHETIC AMORPHOUS SILICA AS A CARRIER IN WHITE SUGAR

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
DouxMatok Ltd.
9 Shimshon Street
Petach-Tikva 49517
Israel

DATE:
25 February 2021

GRAS Notice for Synthetic Amorphous Silica as a Carrier in White Sugar

TABLE OF CONTENTS

List of Appendices

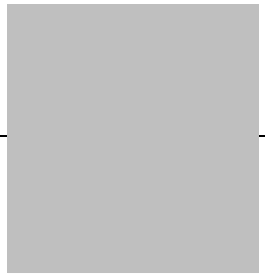
List of Figures and Tables

GRAS Notice for Synthetic Amorphous Silica as a Carrier in White Sugar

Part 1. § 170.225 SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, DouxMatok Inc. (DouxMatok) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended use of synthetic amorphous silica (SAS) as a carrier in sugar is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on DouxMatok's view that these notified food uses are Generally Recognized as Safe (GRAS). In addition, as a responsible official of DouxMatok, the undersigned hereby certifies that all data and information presented in this Notice represent a complete and balanced submission that is representative of the generally available literature. DouxMatok considered all unfavorable, as well as favorable, information that is publicly available and/or known to DouxMatok and that is pertinent to the evaluation of the safety and GRAS status of SAS as described herein.

Signed,



25 February 2021

David Tsivion
VP R&D
DouxMatok Ltd.

Date

1.1 Name and Address of Notifier

DouxMatok Ltd.
9 Shimshon Street
Petach-Tikva 49517
Israel

1.2 Common Name of Notified Substance

The subject of this Notice is food-grade synthetic amorphous silica (SAS) in the form of silica gel.

1.3 Conditions of Use

DouxMatok intends to market SAS as a carrier in white sugar (sucrose). This is based on a proprietary technology that has been developed by DouxMatok for flavor delivery, resulting in an increased perception of sweetness when consumed. The sucrose crystals containing SAS produced using DouxMatok's proprietary technology is referred to as 'DouxMatok Sugar' throughout this Notice. The proposed use levels of SAS in sucrose are provided in Table 1.3-1 below. The food category is organized according to 21 CFR §170.3. It should be noted that neither SAS nor DouxMatox Sugar are intended for use in foods targeted to infants (infant formula) or in meat and poultry products, which would fall under the purview of the U.S. Department of Agriculture.

Table 1.3-1 Individual Proposed Food Use and Use Level for SAS in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food Uses	Silica Use Levels (g/100 g)
Sugar, white, granulated	White sugar	0.05 to 0.30

CFR = *Code of Federal Regulations*; SAS = synthetic amorphous silica; U.S. = United States.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2019), DouxMatok has concluded that the intended use of SAS as described herein is GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

DouxMatok Ltd.
14 Odem Street
Petach-Tikva 49517
Israel

Should the U.S. FDA have any questions or additional information requests regarding this Notification, DouxMatok will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

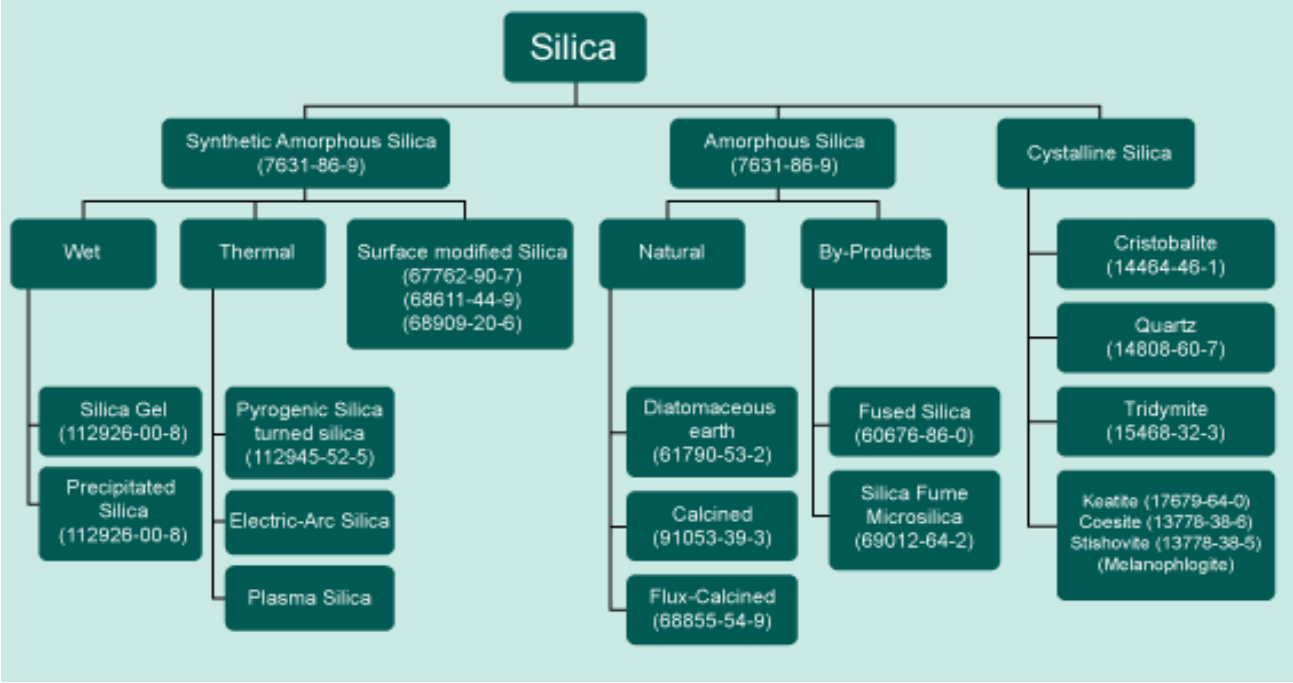
It is DouxMatok's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the *Freedom of Information Act*, 5 U.S.C. 552.

Part 2. § 170.230 IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

2.1 Identity

There are 3 main types of silica (silicon dioxide; SiO_2) defined under Chemical Abstracts Service (CAS) No. 7631-86-9, which include (i) crystalline silica; (ii) amorphous (non-crystalline) silica, which is naturally-occurring or produced as a by-product in the form of fused silica or silica fume; and (iii) synthetic amorphous silica, or SAS (see Figure 2.1-1). There exist various types of SAS, based on whether they are produced through a wet route (precipitated silica or silica gel, also known as hydrated silica or silica aerogel) or a thermal route (pyrogenic silica) (Fruijtier-Poelloth, 2012). Precipitated silica and silica gel are chemically identical (CAS No. 112926-00-8), but possess slightly different physicochemical properties (*e.g.*, pore size distribution; silica gel tends to have a narrower pore size distribution than precipitated silica) (EFSA, 2018). Colloidal silica (silica sol) is a stable dispersion of SAS in a liquid (generally water). The subject of this GRAS Notification is SAS in the form of silica gel.

Figure 2.1-1 Various Forms of Silica



The product specifications for SAS meet the specifications in the silicon dioxide monograph, as outlined in the *Food Chemicals Codex* (FCC) (11th edition) (FCC, 2018). SAS material that is the subject of this GRAS Notification is also chemically identical to the other SAS materials that were considered to be GRAS for their intended uses under GRNs 321 and 554 (U.S. FDA, 2010, 2015). The average particle size of SAS ranges from 4.5 to 5.3 micrometers (µm), as determined by laser light scattering method, suggesting that the silica particles do not fall under the definition of nanoparticles (*i.e.*, particles with a diameter of <100 nm).

2.2 Manufacturing Process

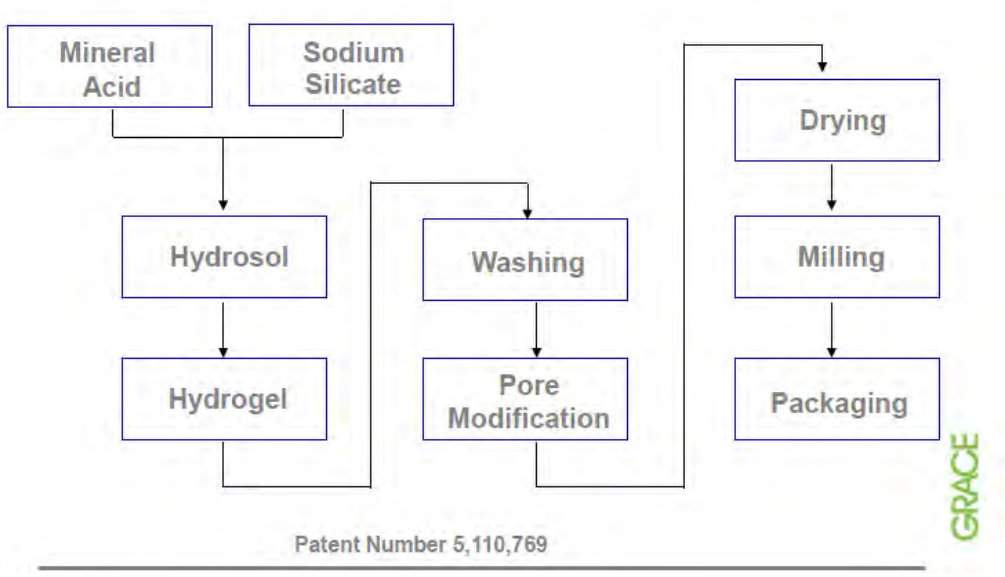
2.2.1 Manufacturing Process of SAS

SAS is manufactured in accordance with current Good Manufacturing Practice (cGMP). Synthetic amorphous precipitated silica and silica gels are manufactured using a wet process that involves an alkali metal silicate solution (also called "water glass") and acids, typically sulfuric acid. Briefly, the process involves precipitation, filtration, washing, drying, milling, and granulation, followed by packing and shipping of the product. For the purposes of producing silica gel, the pH is adjusted from very basic to neutral/slightly acidic using sulfuric acid. The manufacturer of SAS (GRACE) used in the production of DouxMatok Sugar stated:

> "*Silica gels are generally manufactured under acidic conditions with primary particles in the range of 1 to 10 nanometers (nm) that upon drying quickly adhere to form aggregates ranging from 1 to 20 micrometers (µm)*".

The manufacturing process of SAS is presented in Figure 2.2.1-1 below.

Figure 2.2.1-1 Schematic Overview of the Manufacturing Process for SAS



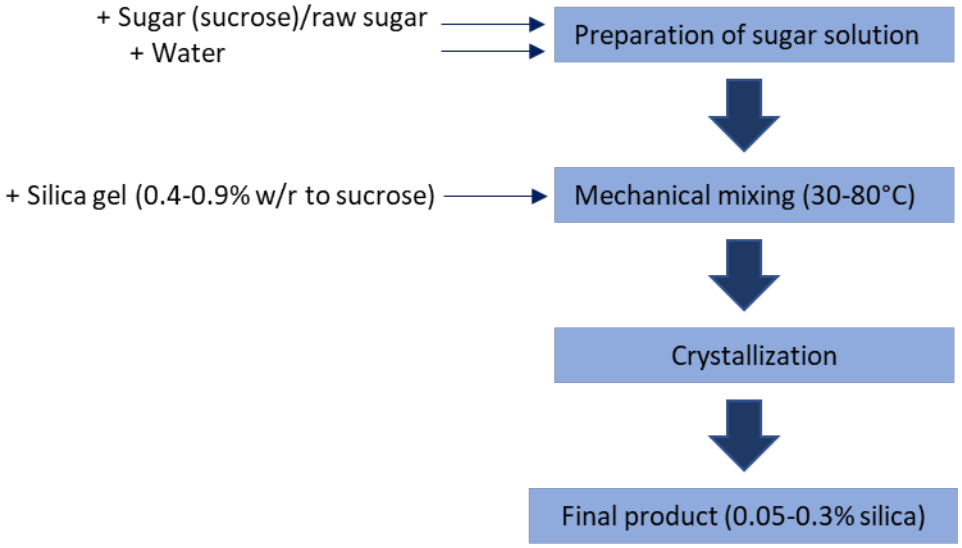
SAS = synthetic amorphous silica.

2.2.2 Manufacturing Process of DouxMatok Sugar

DouxMatok intends to market SAS as a carrier in white sugar (sucrose) at levels described in Table 1.3-1. The process is based on a proprietary technology that has been developed by DouxMatok for flavor delivery by coating/loading food-grade silica particles with various nutritive and non-nutritive sweeteners to form a sweetener/carrier composition through non-covalent interactions (hydrogen and van der Waals), resulting in an increased perception of sweetness when consumed. DouxMatok Sugar is referred to the sucrose crystals containing SAS produced using DouxMatok's proprietary technology.

For the production of DouxMatok Sugar, food-grade SAS in the form of silica gel is mixed mechanically with sucrose using a high shear mixer and then crystalized. No chemical bonds are formed between sugar and SAS (as demonstrated in Section 2.2.3); instead, sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions. A schematic of the manufacturing process is provided in Figure 2.2.2-1 below. The final silica content in the dry sugar crystals is 0.05 to 0.3%.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for DouxMatok Sugar

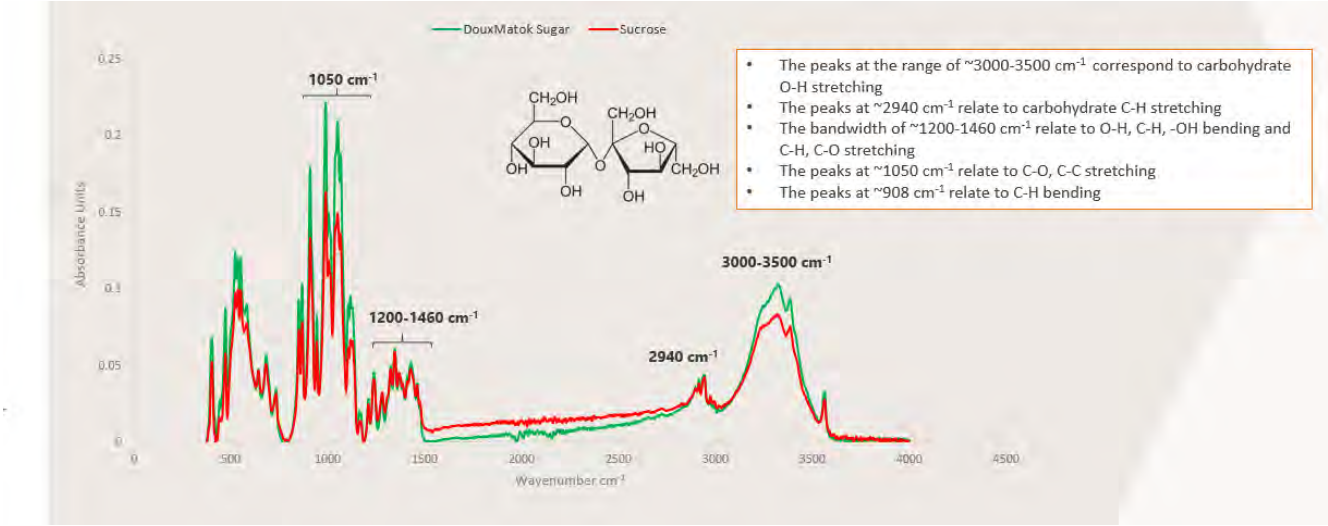


w/r = with respect.

2.2.3 Data Demonstrating Absence of Chemical Interactions between Silica and Sugar in DouxMatok Sugar

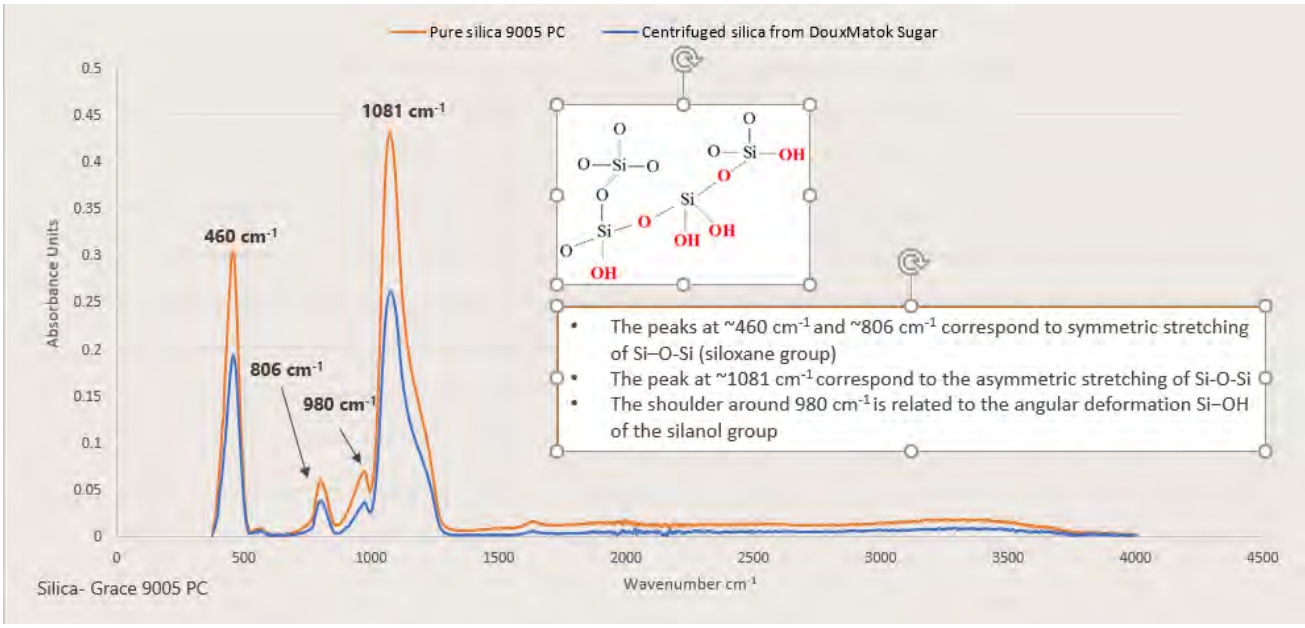
In DouxMatok Sugar, no chemical bonds are formed between the sugar and SAS. Instead, sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions. Using attenuated total reflection in conjunction with Fourier transform infrared (ATR-FTIR) spectroscopy, DouxMatok demonstrated that once in water, sugar and SAS molecules are completely dissociated. Briefly, DouxMatok Sugar was dissolved in water to create a 20 Bx solution. The solution was centrifuged to separate silica. The remaining silica was washed several times with water and the sample was analyzed using ATR-FTIR and compared to SAS, sucrose, and SAS sample separated from DouxMatok Sugar through centrifugation (Figures 2.2.3-1 to 2.2.3-3). As shown in these figures below, there is no evidence for sugar-SAS interactions following DouxMatok Sugar's dissolution in water, indicating that once dissolved DouxMatok Sugar is completely dissociated into its components, SAS and sugar.

Figure 2.2.3-1 A Comparison of the Spectra from DouxMatok Sugar with Sucrose[a]



[a] Based on a method by Svecnjak *et al*. (2011), Gok *et al*. (2015), and Anguebes *et al*. (2016).

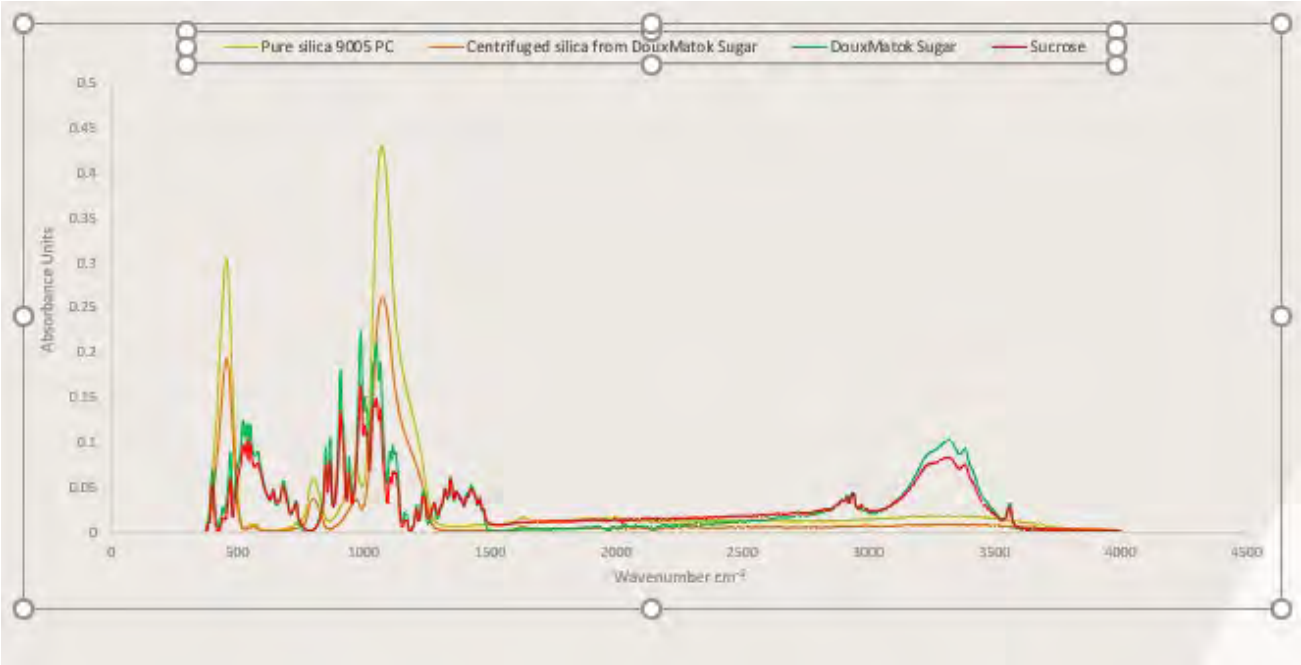
Figure 2.2.3-2 A Comparison of the Spectra from SAS with SAS Separated from DouxMatok Sugar[a]



SAS = synthetic amorphous silica.

[a] Based on a method by Hu and Hsieh (2014) and Rovani *et al*. (2019).

Figure 2.2.3-3 Spectra from Samples of SAS, Sucrose, SAS Separated from DouxMatok Sugar and DouxMatok Sugar



SAS = synthetic amorphous silica.

2.3 Product Specifications and Batch Analysis for SAS

The product specifications for SAS meet the specifications in the silicon dioxide monograph, as outlined in the FCC (11th edition) (FCC, 2018). The average particle size of SAS, which is used in the manufacture of DouxMatok Sugar ranges from 4.5 to 5.3 µm, as demonstrated by laser light scattering technique, suggesting that the silica particles do not fall under the definition of nanoparticles (*i.e.*, particles with a diameter of <100 nm).

Analysis of 5 non-consecutive lots of SAS demonstrates that the manufacturing process, as described in Section 2.2.1, produces a consistent product that meets product specifications. A summary of the chemical analysis for the 5 lots of SAS is presented in Table 2.3-1. All methods used in the analysis are internationally-recognized or validated methods.

Table 2.3-1 Summary of the Chemical Product Analysis for 5 Non-Consecutive Lots of SAS

Specification Parameter	Specification	Method of Analysis	Manufacturing Lot				
			5210199001	5210199004	5210151142	5210181145	5210181147
Appearance	Fine white powder	Visual	Conforms	Conforms	Conforms	Conforms	Conforms
Loss on Ignition (@ 1,832 °F) (%w/w)	≤8.5	USP 733	3.1	3.6	3.3	3.4	2.9
Loss on Drying (@ 105°C) (%w/w)	≤7	USP 731	1.5	3.0	2.4	3.5	2.0
Silica (SO_2) (%w/w)	≥99.4	ICP Validated to USP 232/233	99.6	99.6	99.4	99.6	99.8

Table 2.3-1 Summary of the Chemical Product Analysis for 5 Non-Consecutive Lots of SAS

Specification Parameter	Specification	Method of Analysis	Manufacturing Lot				
			5210199001	5210199004	5210151142	5210181145	5210181147
Average Particle Size (µm)	4.5 to 5.3	Malvern Mastersizer 2000 Laser light scattering technique	4.8	4.9	5.1	5.2	4.6
Heavy metals							
Lead (ppm)	≤4.5	ICP-MS (USP 232/233)	≤4.5	≤4.5	≤4.5	≤4.5	≤4.5
Mercury (ppm)	≤1	ICP-MS (USP 232/233)	≤1	≤1	≤1	≤1	≤1
Total heavy metals (ppm)	≤20	ICP (USP 231)	≤20	≤20	≤20	≤20	≤20
Impurities							
Na_2SO_4 (%)	≤5	ICP (USP 231)	≤5	≤5	≤5	≤5	≤5
Na_2O (%)	≤0.2	ICP (USP 231)	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2
Al2O3 (%)	≤0.2	ICP (USP 231)	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2
Fe2O3 (ppm)	≤400	ICP (USP 231)	≤400	≤400	≤400	≤400	≤400
Microbiological							
Total aerobic plate count (CFU/g)	<500	USP 61	<500	<500	<500	<500	<500
Yeast and mold (CFU/g)	<500	USP 61	<500	<500	<500	<500	<500
Gram negative bacilli (CFU/g)	<10	USP 62	<10	<10	<10	<10	<10
Staphylococcus aureus (CFU/g)	<10	USP 62	<10	<10	<10	<10	<10
Salmonella	Negative	USP 62	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units; ICP = inductively coupled plasma; ICP-MS = inductively coupled plasma mass spectrometry; LOD = limit of detection; ppm = parts per million; SAS = synthetic amorphous silica; USP = United States Pharmacopeia.

2.4 Product Specifications and Batch Analysis for DouxMatok Sugar

Product specifications for DouxMatok Sugar and the results of chemical analysis for 6 lots of DouxMatok Sugar are provided in Table 2.4-1. The analytical methods are consistent with internationally-recognized methods. The average particle size of SAS used in the manufacture of DouxMatok Sugar ranges from 4.5 to 5.3 µm, suggesting that the silica particles do not fall under the definition of nanoparticles (*i.e.*, particles with a diameter of <100 nm)

Table 2.4-1 Summary of the Chemical Product Analysis for 6 Non-Consecutive Lots of DouxMatok Sugar

Specification Parameter	Specification	Method of Analysis	Manufacturing Lot					
			S1LNCRF2	S1LNCRF8	S1LNCRF10	S1SZWCRK14	S1SZWCRK17	S1SZWCRK18
Sucrose (%)	≥98	The Braunschweig Method for the Polarisation of White Sugar by Polarimetry (ICUMSA, 2011a)	99.7	99.7	99.7	99.8	99.6	99.5
Water (%)	≤2	The Determination of Sugar Moisture by Loss on Drying (ICUMSA, 2007a)	0.02	0.02	0.04	0.07	0.07	0.07
Ash (conductivity) – non silica (%)	≤2	The Determination of Conductivity Ash in Refined Sugar Products and in Plantation White Sugar (ICUMSA, 2011b)	0.01	0.02	0.01	0.008	0.013	0.013
Silica (%)	<0.3	The Determination of Conductivity Ash in Refined Sugar Products and in Plantation White Sugar (ICUMSA, 2011b)	0.05	0.084	0.072	0.092	0.107	0.267
Particle Size <3 mm (%)	≥99	The Determination of the Particle Size Distribution of White Sugar and Plantation White Sugar by Sieving (ICUMSA, 2007b)	100	100	100	100	100	100

Part 3. §170.235 DIETARY EXPOSURE

3.1 Functionality

The purpose of embedding the SAS in the sugar is to improve the delivery of sucrose, and increasing its rate of dissolution. The process is based on a proprietary technology (S1 Technology) that has been developed by DouxMatok for flavor delivery by coating/loading food-grade silica particles with various nutritive and non-nutritive sweeteners to form a sweetener/carrier composition through non-covalent interactions (hydrogen and van der Waals). This would result in an increased perception of sweetness when consumed, thereby significantly reducing the amount of sugar needed to produce a desired level of sweetness in a food. The use of SAS in white sugar is consistent with the definition of a 'carrier', as defined by the Codex as:

> "*A food additive used to dissolve, dilute, disperse or otherwise physically modify a food additive or nutrient without altering its function (and without exerting any technological effect itself) in order to facilitate its handling, application or use of the food additive or nutrient*" (Codex, 2018).

The sucrose crystals containing SAS produced using the S1 technology is referred to as 'DouxMatok Sugar'. The final silica content in the dry sugar crystals is 0.05 to 0.3%

3.2 Estimated Dietary Intake of Silica from Proposed Food Uses

3.2.1 Methodology

An assessment of the anticipated intake of SAS under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2018a,b; USDA, 2018). A summary of the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2015-2016. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2018a,b; USDA, 2018). The NHANES data were employed to assess the mean and 90th percentile intake of SAS for each of the following population groups:

- Infants and toddlers, less than 2 years of age;
- Young children, ages 2 to 5;
- Children, ages 6 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (ages 2 years and older, and both gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of SAS by the U.S. population[1]. Estimates for the daily intake of SAS represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of SAS averaged over all individuals surveyed, regardless of whether they consumed food products in which SAS is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing SAS during the 2 survey days). "Consumer-only" intake refers to the estimated intake of SAS by those individuals who reported consuming food products in which the use of SAS is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which SAS is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of SAS were generated using the maximum use level indicated for the intended food uses, as presented in Table 1.3-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for these assessments are presented in Section 3.3.

3.3 Results of Intake Estimates for SAS

A summary of the estimated daily intake of SAS from the proposed food use of white sugar is provided in Table 3.3-1 on an absolute basis (mg/person/day), and in Table 3.3-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was evaluated among the total population (*i.e.*, 2 years and older) and among individual population groups in the current intake assessment; greater than 20.6% of the individual population groups consisted of consumers of food products in which SAS is currently proposed for use (Table 3.3-1). Female adults had the greatest proportion of consumers at 53.5%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (2 years and older), the mean and 90th percentile consumer-only intakes of SAS were determined to be 46 and 103 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of SAS on an absolute basis, at 53 and 138 mg/person/day, respectively, while infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 12 and 18 mg/person/day, respectively (Table 3.3-1).

Table 3.3-1 Summary of the Estimated Daily Intake of SAS from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Toddlers	0 to <2	2	3	20.6	87	12	18
Young Children	2 to 5	6	20	37.8	212	17	35
Children	6 to 11	10	27	48.5	398	21	50

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.3-1 Summary of the Estimated Daily Intake of SAS from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Female Teenagers	12 to 19	20	57	49.7	210	41	84
Male Teenagers	12 to 19	15	45	38.2	189	40	90
Female Adults	20 and older	25	69	53.5	1,302	47	106
Male Adults	20 and older	27	72	51.3	1,083	53	138
Total Population	2 and older	23	59	50.4	3,394	46	103

n = sample size; NHANES = National Health and Nutrition Examination Survey; SAS = synthetic amorphous silica; U.S. = United States.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of SAS were determined to be 0.65 and 1.52 mg/kg body weight/day, respectively. Among the individual population groups, infants and toddlers were identified as having the highest mean consumer-only intakes of any population group, of 1.01 mg/kg body weight/day, while young children had the highest 90th percentile intake estimate of 2.06 mg/kg body weight/day. Male teenagers had the lowest mean and 90th percentile consumer-only intakes of 0.59 and 1.41 mg/kg body weight/day, respectively (Table 3.3-2).

Table 3.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of SAS from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Toddlers	0 to <2	0.21	0.29	20.6	87	1.01	1.84
Young Children	2 to 5	0.37	1.33	37.9	210	0.99	2.06
Children	6 to 11	0.32	0.86	48.5	397	0.66	1.57
Female Teenagers	12 to 19	0.33	1.07	49.8	206	0.66	1.64
Male Teenagers	12 to 19	0.23	0.71	38.4	189	0.59	1.41
Female Adults	20 and older	0.34	0.91	53.5	1,293	0.64	1.49
Male Adults	20 and older	0.33	0.81	51.4	1,072	0.64	1.56
Total Population	2 and older	0.33	0.91	50.5	3,367	0.65	1.52

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; SAS = synthetic amorphous silica; U.S. = United States.

3.4 Summary and Conclusions

Consumption data and information pertaining to the intended food uses of SAS were used to estimate the *per capita* and consumer-only intakes of SAS for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment, rendering exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain SAS at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that SAS will have 100% market penetration in the food category of white sugar.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of SAS by the total U.S. population from proposed food uses in the U.S., were estimated to be 46 mg/person/day (0.65 mg/kg body weight/day) and 103 mg/person/day (1.52 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of SAS were determined to be 53 mg/person/day (0.64 mg/kg body weight/day), and 138 mg/person/day (1.56 mg/kg body weight/day), respectively, as identified among male adults. While infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 12 and 18 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest mean daily intake of 1.01 mg/kg body weight/day while young children had the highest 90th percentile intake estimate of 2.06 mg/kg body weight/day. It should be noted that neither SAS nor DouxMatox Sugar are intended for use in foods targeted to infants (infant formula) or in meat and poultry products, which would fall under the purview of the U.S. Department of Agriculture.

Part 4. §170.240 SELF-LIMITING LEVELS OF USE

Use of SAS at higher levels in food applications (*e.g.*, >4%) is associated with reduced sweetness and an undesired mouthfeel. As such, the use levels of SAS are self-limiting.

Part 5. §170.245 EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Not applicable.

Part 6. §170.250 NARRATIVE AND SAFETY INFORMATION

6.1 Safety Narrative

The food-grade SAS intended for use as a carrier in DouxMatok Sugar is mixed mechanically with sucrose and then dried (typically, in a crystallization process) to form the final dry product. Throughout this process, no chemical bonds are formed between sugar and SAS molecules (as demonstrated in Section 2.2.3); instead, sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions. Considering the absence of any chemical interactions between SAS and sucrose and given that SAS discussed herein is chemically representative of the SAS materials that were previously concluded to be GRAS (*i.e*., GRNs 321 and 554), a discussion of publicly available data and information relevant to the safety of SAS is incorporated by reference to pivotal studies discussed in GRNs 321 and 554.

The use of SAS, as a direct and indirect food ingredient, has been concluded to be GRAS at levels up to 2% in the finished food product; these GRAS conclusions were notified to the U.S. FDA and filed by the Agency under GRNs 321 and 554 and received "no questions" letter (U.S. FDA, 2010, 2015). The GRAS uses of SAS; however, do not include use in white sugar or sucrose, and currently, there are no regulatory provisions permits for the explicit use of silica as a carrier in white sugar.

As previously demonstrated in Section 2.2.3, the interactions between the sugar and SAS molecules in DouxMatok Sugar are of *via* hydrogen and van der Waals bonds, and thus, no chemical interactions between the two molecules are formed. Spectral data using ATR- FTIR spectroscopy demonstrated that there are no sugar-SAS interactions following DouxMatok Sugar's dissolution in water, indicating that once dissolved DouxMatok Sugar is completely dissociated into its components, SAS and sugar. Considering this, the safety of SAS for use in DouxMatok Sugar can be assessed on the basis of the safety of its components.

The SAS used in the production of DouxMatok Sugar is chemically representative of the SAS materials that were previously concluded to be GRAS (*i.e*., GRNs 321 and 554), accordingly, a discussion of publicly available data and information relevant to the safety of SAS is incorporated by reference to pivotal studies discussed in GRNs 321 and 554. SAS in DouxMatok Sugar is used up to a level that is well below the levels of SAS previously concluded to be GRAS (0.3% *versus* 2%). Additionally, SAS used in the production of DouxMatok Sugar, similar to the materials in GRNs 321 and 554, does not fall under the definition of nanomaterials (particles with a diameter <100 nm), as demonstrated by the manufacturer of SAS through laser light scattering technique (see Section 2.3). This safety evaluation does not include assessment of nano-SAS. Sucrose is affirmed as GRAS under §184.1854 for addition to all foods at levels consistent with cGMP (U.S. FDA, 2020).

The GRAS conclusions in GRNs 321 and 554 were on the basis of scientific procedures, supported by the publicly available data evaluated by various organizations, including the Select Committee on GRAS Substances (SCOGS) (FASEB, 1979), the Organisation of Economic Co-operation and Development (OECD) Screening Information Data Sets program (OECD SIDS, 2005), and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (ECETOC, 2006). The safety of SAS as a food additive was the subject of evaluations by various authoritative bodies, including the European Food Safety Authority (EFSA), the Scientific Committee on Food (SCF), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1969, 1974a,b, 1982; SCF, 1991; EFSA, 2018).

To identify new data pertinent to the safety of SAS published since the GRAS status of SAS was last evaluated in 2014 (*i.e*., GRN 554), a comprehensive search of the published scientific literature was conducted for the period spanning from June 2014 through August 2020. The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Based on this updated search of the literature, DouxMatok is not aware of any newly published studies that suggest that SAS would be unsafe when used as a food ingredient.

A summary of the pertinent toxicological studies from prior GRAS Notifications and newly identified studies or publicly available scientific evaluation relevant to the safety of SAS is provided in the sections that follow. Based on conclusions from previous expert panels on the GRAS status of SAS, corresponding "no questions" letters issued by the U.S. FDA for GRNs 321 and 554 (U.S. FDA, 2010, 2015), the widespread history of use of SAS as a food additive globally, and conclusions from other authoritative and scientific bodies on the safety of SAS (*e.g*., SCOGS, OECD, JECFA, ECETOC, EFSA, and SCF), DouxMatok has concluded that the current GRAS status of SAS, as described in GRNs 321 and 554, can be extended to SAS used in the manufacture of DouxMatok Sugar. DouxMatok has therefore concluded that SAS, as described herein, is GRAS, for use as a carrier in the production of DouxMatok Sugar, based on scientific procedures.

The available data related to the safety of SAS are summarized below.

6.2 Metabolic Fate

The metabolic pathway of SAS has been evaluated as part of various safety assessments conducted by SCOGS, the OECD, and the ECETOC (FASEB, 1979; OECD SIDS, 2005; ECETOC, 2006) and GRNs 321 and 554 (U.S. FDA, 2010, 2015), and more recently as part of EFSA's re-evaluation of silicon dioxide as a food additive (EFSA, 2018). The results of animal and human studies indicate that, following ingestion, SAS is not expected to undergo significant intestinal absorption and any small quantities of soluble SAS that is absorbed will be excreted unchanged in the urine. Accumulation in body tissues was reported to be limited with no indication of metabolism of SAS. The ECETOC (2006) report concluded that, "*In contrast to crystalline silica, SAS is soluble in physiological media and soluble chemical species are formed which are eliminated via the urine without modification after intestinal resorption*".

6.3 Safety Evaluations of SAS

Silicon dioxide is a food additive that is permitted for direct addition to foods intended for human consumption (21 CFR §172.480 – U.S. FDA, 2019). The U.S. FDA issued a "no questions" letter to the Notification of the GRAS status of the use of SAS as an anticaking agent, defoaming agent, stabilizer, adsorbent, carrier, conditioning agent, chill proofing agent, filter aid, emulsifying agent, viscosity control agent, and anti-settling agent in a variety of food categories (GRN 321 – U.S. FDA, 2010). The Notification of the GRAS status of SAS also received a "no questions" letter for use as a multi-functional direct ingredient in a broad range of food categories at levels not to exceed 2% of the finished food, and also as an indirect ingredient in the manufacture of adhesives, coatings, defoaming agents, greases and lubricants, paper and paperboard, and polymers used as components of food-packaging material (GRN 554 – U.S. FDA, 2015). Additionally, silica gel or silica aerogel, defined as a finely powdered microcellular silica foam having a minimum silica content of 89.5%, is GRAS when used as a component of an antifoaming agent in accordance with cGMP) (21 CFR §182.1711 – U.S. FDA, 2019).

The safety of various forms of SAS has been extensively reviewed by a number of authoritative and scientific bodies including EFSA, SCF, and JECFA (JECFA, 1969, 1974a,b, 1982; SCF, 1991; EFSA, 2018). A summary of the safety studies related specifically to silica gel is provided in Section 6.3.1 to 6.3.3, as described below.

6.3.1 Genotoxicity

A summary of genotoxicity studies conducted with silica gel form of SAS, as reviewed by the authoritative and scientific bodies, is provided in Table 6.3.1-1 below. In the most recent review by an authoritative and scientific body, the EFSA Panel concluded that:

> "*For SAS used as a food additive, in cosmetics or in pharmaceuticals, the available in vitro and in vivo study results, although of 'limited relevance' did not indicate any potential for genotoxicity, and overall the Panel considered that SAS used as a food additive did not raise a concern with respect to genotoxicity*".

Table 6.3.1-1 Summary of Genotoxicity Studies for SAS – Silica Gel

Test	Test System/ Animal Species	Test Substance (Trade Name)	Concentration/Dose	Results	Reference
In Vitro					
Bacterial reverse mutation assay	Salmonella Typhimurium TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2	Silica gel (Silcron G-910)	Up to 10,000 µg/plate (±S9)	Negative	ECETOC (2006); EFSA (2018)
Bacterial reverse mutation assay	S. Typhimurium TA1530, G-46	Precipitated silica gel, crystalline-free (Syloid 244)	NR (-S9)	Negative	OECD SIDS (2005); ECETOC (2006)
Chromosomal aberration assay	Human embryonic lung cells (Wi-38)	Precipitated silica gel, crystalline-free (Syloid 244)	1 to 1,000 µg/mL (-S9; 24 h)	Negative	ECETOC (2006); EFSA (2018)
Gene mutation assay	*Saccharomyces cerevisiae* D3	Precipitated silica gel, crystalline-free (Syloid 244)	NR (-S9)	Negative	OECD SIDS (2005); ECETOC (2006)
Cytogenic assay	Human embryonic lung cells	Precipitated silica gel, crystalline-free (Syloid 244)	1 to 1,000 µg/mL (metabolic activation NR)	Negative	OECD SIDS (2005)
Single-cell gel/Comet assay	Human embryonic lung cells	Silica gel (Spherisorb)	17.2 to 137.9 µg/mL (-S9; 3 h)	Positive (significant DNA migration ≥68.9 µg/mL)	OECD SIDS (2005); ECETOC (2006); EFSA (2018)
Single-cell gel/Comet assay	Chinese hamster lung (V79) cells	Silica gel (Spherisorb)	17.2 to 137.9 µg/mL (-S9; 3 h)	Positive (significant DNA migration ≥68.9 µg/mL)	OECD SIDS (2005); ECETOC (2006); EFSA (2018)
Micronucleus test	Chinese hamster lung (V79) cells	Silica gel (Spherisorb)	20 to 160 µg/mL (-S9; 24 h)	Positive (weak but significant induction of micronuclei)	OECD SIDS (2005); ECETOC (2006); EFSA (2018)
In Vivo					
Chromosomal aberration test	Rat (Sprague-Dawley) M (5/group) Oral (gavage)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg Repeat-dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg 5 times per day	Negative	OECD SIDS (2005)

Table 6.3.1-1 Summary of Genotoxicity Studies for SAS – Silica Gel

Test	Test System/ Animal Species	Test Substance (Trade Name)	Concentration/Dose	Results	Reference
Dominant lethal assay	Rat (Sprague-Dawley) M, F (number of animals per group NR) Oral (gavage)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg Repeat-dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg 5 times per day	Negative	OECD SIDS (2005); ECETOC (2006); EFSA (2018)
Gene mutation assay (host-mediated)	Mouse (strain NR) and S. Typhimurium TA1530, G-46 (indicator)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4 to 5,000 mg/kg Repeat-dose: 1.4 to 5,000 mg/kg; 5 times per day	Negative	ECETOC (2006)

-S9 = in the absence of metabolic activation; +S9 = in the presence of metabolic activation; F = female animals; h = hour(s); M = male animals; NR = not reported; SAS = synthetic amorphous silica.

6.3.2 Preclinical Safety

A summary of preclinical studies conducted with silica gel from of SAS, as reviewed by various authoritative and scientific bodies is provided in Table 6.3.2-1 below. No adverse effects were reported in rats after receiving silica gel for 6 months at dose levels of 0, 3.2 or 10% in the diet (equal to 0, 2,170, or 7,950 mg/kg body wight/day in males and 0, 2,420, or 8,980 mg/kg body weight/day per day in females). No adverse or carcinogenic effects were reported in rats after receiving dietary doses of 0, 625, 1,250, or 2,500 mg/kg body weight/day in rats and 1,875, 3,750, or 7,500 mg/kg body weight/day in mice, the highest doses tested.

Table 6.3.2-1 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Subchronic Studies						
Rat (CD-1) M, F (12/sex/group)	Oral (diet) 6 months	Precipitated silica gel, crystalline-free (Syloid 244)	M: 0, 2,170, or 7,950 F: 0, 2,420, or 8,980 (0, 3.2, or 10%)	Physical appearance Food consumption Growth Survival Hematology Clinical chemistry Blood chemistry Urinalysis Macroscopic examination Histology examination	• No treatment-related findings in any measured parameters • No effects on physical appearance, food consumption, growth, or survival • No clinical signs or effects on behavior and body weights observed • No effects on clinical chemistry observed • No histopathological changes observed in kidneys • NOAEL = 8,980 mg/kg bw/day	OECD SIDS (2005); ECETOC (2006); EFSA (2018)
Rat (Wistar) 100 M	Oral (diet) 18 weeks	Sodium metasilicate	0, 100, 200, or 400 (0, 0.05, 0.1, or 0.2%)	NR	• No adverse effects reported	EFSA (2009)
Rat (Sprague Dawley) 5 M, 5 F	Oral (diet) 2 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	<u>Day 1 to 10:</u> 0, 5,800, or 16,500 (0, 5, or 10%) <u>Day 11 to 14:</u> 24,200 (20%)	Clinical symptoms Food consumption Water consumption Body weight gain Behavior	• No clinical symptoms observed • No effects on food or water consumption, body weight gain, or behavior observed	ECETOC (2006)
Rat (strain NR) 10 M/group	Oral (diet) 28 days	Micronized silica gel	0, 0.2, 1.0, or 2.5%	Mortality Abnormal gross autopsy Body weight gain	• No adverse effects observed • No mortality • No abnormal necropsy findings were observed • ↓ body weight gain [1.0, 2.5]	JECFA (1969); EFSA (2004)

Table 6.3.2-1 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Reproductive and Developmental Toxicity						
Rat (Wistar) 20 to 25 F	Oral (gavage) GD 6 to 15	Precipitated silica gel, crystalline-free (Syloid 244)	0, 14, 63, 290, or 1,350	Clinical signs Survival Maternal body weight Abnormalities (soft and skeletal tissues)	F_0 • No effect on nidation or maternal/fetal survival • No maternal toxicity reported • NOAEL = 1,350 mg/kg bw/day F_1 • Soft and skeletal tissue abnormalities did not differ between treatment and sham-treated control groups • No developmental toxicity reported • NOAEL = 1,350 mg/kg bw/day	OECD SIDS (2005); ECETOC (2006); EFSA (2009, 2018)
Mouse (CD-1) 21 to 24 F	Oral (gavage) GD 6 to 15	Precipitated silica gel, crystalline-free (Syloid 244)	0, 13, 62, 290, or 1,340	Clinical signs Survival Number of abortions Live Litters Implantation sites Resorptions Dead and live fetuses Fetal weight Abnormalities (soft and skeletal tissues)	F_0 • No effect on nidation or maternal/fetal survival • ↓ maternal weight GD 15 and 17 [1,340] (no statistical evaluation conducted) • *"The relevance of this finding was questionable since the initial weight of dams in this group at GD 0 was 8% lower than in controls"* (EFSA, 2018) • NOAEL = 1,350 mg/kg bw/day F_1 • ↓ bw and skeletal retardation [1,340] (no statistical evaluation conducted) • Soft and skeletal tissue abnormalities did not differ • NOAEL = 1,350 mg/kg bw/day • "*The panel considered that in the absence of statistical evaluation the biological relevance of the reported changes cannot be evaluated*" (EFSA, 2018)	ECETOC (2006); EFSA (2009, 2018); OECD SIDS (2005)

Table 6.3.2-1 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Hamster (Syrian golden)	Oral (gavage) GD 6 to 10	Silica gel (Syloid 244)	0, 16, 74, 345, or 1,600	Clinical signs Maternal body weight	• No maternal or developmental toxicity observed	EFSA (2009, 2018)
Rabbit (Dutch-belted)	Oral (gavage) GD 6 to 18	Silica gel (Syloid 244)	0, 16, 74, 345, or 1,600	Clinical signs Maternal body weight Fetal weight Number of abortions Live litters Corpora lutea Implantation sites Early and late resorptions Dead and live fetuses Sex ratio External abnormalities Post-natal survival	• No differences in post-natal survival, abortions, and body weight gain during pregnancy • No dose-response effect observed in the ↑ of dead fetuses • ↓ average fetal weight [1,600] (no statistical evaluation conducted) • No developmental abnormalities observed • "*The Panel considered that in this study the documentation of data and the number of litters for fetopathological examination were not sufficient to reach a final conclusion*" (EFSA, 2018)	EFSA (2009, 2018)
Carcinogenicity						
Rat (Fischer 344) M, F (40/sex/group)	Oral (diet) 103 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	0, 625, 1,250, or 2,500 (0, 1.25, 2.5, or 5%)	Clinical sings Food consumption Survival Body weight Hematology Clinical chemistry Organ weight Tumor incidence	• No clinical signs observed • NS variations in survival rats (M, F) • No effects on body weight and food consumption • No treatment-related effects on hematology and clinical chemistry • ↓ liver weight from 12 to 24 months (F) [1,250; 2,500] (significance NR) • ↑ tumor incidence in testes and prepuce (M) (group and significance NR) • No pathologic or carcinogenic effects observed • NOAEL = 2,500 mg/kg bw/day	EFSA (2004, 2009, 2018); OECD SIDS (2005); ECETOC (2006)

Table 6.3.2-1 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Mouse (B6C3F1) M, F (40/sex/group)	Oral (diet) 93 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	0, 1,875, 3,750, or 7,500 (0, 1.25, 2.5, or 5%)	Clinical signs Hematology Blood chemistry Urinalysis Gross examination Microscopic examination	• No clinical signs observed [up to 7,500] • ↑ food consumption (M, F) [3,750; 7,500], ↓ body weight gain between Weeks 15 to 50 (M) and Weeks 30 to 50 (F) [7,500] • "*No effects of toxicological relevance on body weight (difference compared with control <10%) and food consumption*" (EFSA, 2018) • NSD in survival rats or behavior observed • No dose-related effects in hematologic parameters • No sex- or dose-related effects in organ weights • No pathologic or carcinogenic effects observed • NOAEL = 7,500 mg/kg bw/day	EFSA (2004, 2009, 2018); OECD SIDS (2005); ECETOC (2006)

bw = body weight; F = female animals; F_0 = parental generation; F_1 = first filial generation; GD = Gestation Day; M = male animals; n = number of animals; NOAEL = no-observed-adverse-effect level; NR = not reported; NS = no significant; NSD = no significant difference; SAS = synthetic amorphous silica.

[a] Unless stated otherwise, all reported effects are statistically significantly different relative to control group(s).

[b] Information in [ ] indicates the dose at which effects were observed.

[c] The Panel considered that in the absence of statistical evaluation, the biological relevance of the reported changes cannot be evaluated.

6.3.3 Human Data

Toxicological studies assessing the safety of SAS in humans following oral exposure were not identified in the extensive search of the literature. No reports were identified with respect to human toxicity following intake of silicon that occurs naturally in food (EFSA, 2004). Low levels of amorphous silicates are also present in human food as a food additive for the purposes of anti-foaming and anti-caking and have been consumed by humans for decades with no reported adverse effects (EFSA, 2004). SAS is also not listed as a carcinogen to humans following an evaluation by the International Agency for Research on Cancer (IARC) (ECETOC, 2006).

Although safety data of SAS in humans is lacking, a few studies following oral exposure of SAS were identified. In 1 study, silica gel was provided to 6 adults with primary type II hyperlipoproteinemia at a dose of 1,000 mg/day twice per day which increased to 16,000 mg/day twice per day by the end of the 3-week of administration and no marked adverse effects were reported and the silica gel did not increase excretion of bile acid (ECETOC, 2006; EFSA, 2018). Single doses of 50 and 2,500 mg silicon dioxide were ingested by adult volunteers and the test article was well-tolerated and was excreted in the urine with no apparent accumulation in the tissues (JECFA, 1974b; OECD SIDS, 2005). Following oral administration of 60 to 100 g daily of 12% amorphous silicic acid to adults with either gastritis or enteritis for 3 to 4 weeks, the test article was well-tolerated and no adverse effects were reported (JECFA, 1969, 1974b).

6.4 Other Considerations Related to the Safety of SAS

6.4.1 Evaluations by Authoritative and Scientific Bodies

6.4.1.1 Tolerable Upper Intake Level for Elemental Silicon

The nutritional role of elemental silicon has been considered by a number of authoritative bodies, including SCF of the European Commission, EFSA, the United Kingdom Expert Group on Vitamins and Minerals (EVM), and the Institute of Medicine of the United States National Academies of Sciences (IOM). Data relevant to the safety of silicon was also critically evaluated by these authoritative bodies during their derivation of a tolerable upper intake level (UL)[2] for elemental silicon.

There was no reported evidence of silica-induced tumors in a 2-year carcinogenicity study in rats and mice, in which amorphous silica was administered as a dietary admixture at dietary levels of 0, 1.25, 2.5, and 5%, to both rats and mice corresponding to theoretical doses of 2,500 mg/kg body weight/day and up to 7,500 mg/kg body weight/day, respectively, without any evidence of silica-induced tumors (Takizawa *et al.*, 1988). Following its critical evaluation of this study, the IOM concluded that typical levels of silicon intake from the diet would not pose any safety concerns for the general population (IOM, 2001). Using consumption data from dietary surveys conducted in the U.S., the IOM estimated that the median intake of elemental silicon for adult men and women ranged from approximately 14 to 21 mg/day (depending on the age category). Additionally, the IOM estimated the median intake of elemental silicon from dietary supplement sources in adults in the U.S. to be approximately 2 mg/day. IOM concluded, "*Due to lack of data indicating adverse effects of silicon, it is not possible to establish a UL*" (IOM, 2001).

[2] EFSA uses the term "tolerable upper intake level" (UL) to refer to the maximum level of total chronic daily intake of a nutrient (from all sources) that is judged to be unlikely to pose a risk of adverse health effects to humans. The EVM uses the term "safe upper level" (SUL) for this same concept (*i.e.*, the SUL represents an intake that can be consumed daily over a lifetime without significant risk to health on the basis of available evidence).

The EVM considered the feeding study in rats and mice conducted by Takizawa *et al.* (1988) to be the key study to derive a safe upper level (SUL) for daily supplemental intake of elemental silicon (EVM, 2003). The no-observed-adverse-effect levels were reported to be 50,000 ppm of supplemental silicon dioxide (2,500 mg/kg body weight/day in rats and 7,500 mg/kg body weight/day in mice), the highest concentration tested in the study. A dose of 2,500 mg silicon dioxide/kg body weight/day is equivalent to approximately 1,165 mg elemental silicon/kg body weight/day. After applying uncertainty factors of 10 for interspecies variations and 10 for interindividual variations, a safe upper level intake of 12 mg elemental silicon/kg body weight/day was derived, which is equivalent to approximately 700 mg/day of elemental silicon for a 60-kg individual.

The Scientific Panel on Dietetic Products, Nutrition, and Allergies (NDA Panel) of EFSA acknowledged occurrence of kidney lesions reported in some animal studies following administration of silicates (EFSA, 2004). Due to the lack of suitable data to support a dose-response relationship for adverse effects following the intake of elemental silicon, a UL could not be derived. The EFSA NDA Panel concluded that based on its long history of safe consumption, the estimated typical dietary intake level of silicon (*i.e.*, 20 to 50 mg/day, corresponding to 0.3 to 0.8 mg/kg body weight/day for a 60-kg individual) is unlikely to cause any adverse effects (EFSA, 2004).

Among the total population, the mean and 90th percentile consumer-only intakes of silica were determined to be 45 and 102 mg/day, respectively (see Table 3.3-1). These silica intakes correspond to silicon intakes of approximately 21 and 48 mg/day at the mean and 90th percentile, respectively. The intakes of elemental silicon from the proposed uses of silica in DouxMatok Sugar are in the range of typical dietary intakes of silicon (*i.e.*, 20 to 50 mg/day), as reported by EFSA (2004).

6.4.1.2 Safety Evaluation by the Select Committee on GRAS Substances (SCOGS)

The SCOGS conducted a comprehensive review of the use, exposure, and safety of silica and silicates in 1979 and stated that silicon dioxide and various silicates occur abundantly in practically all natural waters, animals, and plants, and thus are part of the normal human diet (FASEB, 1979). Silicon compounds that are used as direct food ingredients, with the exception of potassium and sodium silicates, are insoluble or very slightly soluble in water and appear to be biologically inert. The SCOGS also recognized that renal toxicity was reported in some animal studies following the ingestion of sodium silicate, magnesium trisilicate, and finely ground quartz. These effects were substantiated and were suggested to be species-specific. The SCOGS further noted that magnesium trisilicate, the predominant silicate added to foods in the U.S., is recognized as safe for use in large quantities as a component of antacid medicines in humans (FASEB, 1979).

6.4.1.3 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA first evaluated the safety of SAS (INS No. 551) and certain silicates (*i.e.*, aluminum, calcium, magnesium, and sodium alumina silicates) in 1969, and it was concluded that the use of these materials do not need to be limited, provided they are used in amounts consistent with Good Manufacturing Practice (GMP) (JECFA, 1969). The safety of these compounds was re-evaluated by JECFA in 1973 as additional data became available, and a group acceptable daily intake (ADI) level of "not limited"[3] was established for silicon dioxide and aluminum, calcium, and sodium aluminosilicates (JECFA, 1974a,b). The Committee indicated that the available data support the "biological inertness" of orally administered silica and silicates (JECFA, 1969, 1974b). Even though additional data were not available by the meeting of 1982, the temporary qualifier for the ADI of "not limited/specified" was removed at this time, because the Committee decided to revise the specifications for magnesium silicate to exclude magnesium trisilicate (JECFA, 1982).

6.4.1.4 Scientific Committee on Food (SCF)

A safety assessment for the use of SAS (E 551) as a food additive was first conducted by the SCF in 1990 (SCF, 1991). Similar to the conclusions made by JECFA, the SCF established a group ADI level of "not specified" for SAS (E 551) and certain silicates (*i.e.*, sodium, potassium, calcium, and magnesium silicates[4]) when used as anticaking agents (SCF, 1991). Magnesium trisilicate was reported to have a history of safe use as an antacid in humans, without any adverse effects reported (SCF, 1991).

6.4.1.5 The European Food Safety Authority (EFSA)

In addition to its technological functions, SAS (E 551) may also serve nutritive purposes by acting as a source of elemental silicon. In 2009, EFSA's Scientific Panel on Food Additives and Nutrient Sources Added to Food published an opinion regarding the addition of calcium silicate, silicon dioxide, and silicic acid gel for nutritional purposes to food supplements (EFSA, 2009). The Panel concluded that the proposed use levels of silicon dioxide in food supplements, which would provide up to 700 mg/day of elemental silicon, would not pose any safety concerns based on the available toxicity data. Upon re-evaluating the safety of silicon dioxide in 2017, for use as a food additive, the Panel concluded that from the available database there was no indication for toxicity of SAS (E 551) at the reported uses and use levels, but the Panel was unable to confirm the current ADI of "not specified". This was due to limitations reported in the toxicological database, more specifically, with respect to insufficient characterization of particle size distribution, and accordingly, recommended some modifications of the European Union specifications for E 551 (EFSA, 2018).

[3] The ADI level of "not limited" established for silicon dioxide and certain silicates (aluminium, calcium, and sodium aluminosilicates) was subsequently reworded to "not specified" in 1985 (JECFA, 1986). The JECFA has replaced the term "not limited" with "not specified" to describe an ADI level for food substances of very low toxicity. In such cases, based on the available toxicological, biochemical, and clinical data, the total daily intake of the substance arising from its natural occurrence and/or its present use or uses in foods at the levels necessary to achieve the desired technological effect, will not represent a hazard to health. Any additive allocated an ADI of "not specified" must be used in accordance with GMP (*i.e.*, it is technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance).

[4] Silicates containing aluminum are required to comply with the Provisional Tolerable Weekly Intake (PTWI) established for aluminum of 7 mg/kg body weight (SCF, 1991).

6.4.1.6 European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)

Following a comprehensive review of the safety of SAS, the ECETOC Joint Assessment of Commodity Chemicals program reported that the results of several acute oral toxicity studies with various hydrophilic and hydrophobic types of SAS in rats and mice indicate a very low order of toxicity; no deaths and no signs of toxicity were reported at doses up to 5,000 mg SiO_2/kg body weight (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). A number of repeated-dose toxicity studies were reported with SAS in rats confirming an absence of significant toxicity for SAS by oral routes of exposure. More specifically, in a 13-week feeding study, the toxicity of a precipitated SAS product (trade name, SIPERNAT® 22) was evaluated in Wistar rats, receiving the material at 0 (control), 0.5, 2, and 8% of the diet, corresponding to theoretical intakes of 0, 250, 1,000, and 4,000 mg/kg body weight/day. Among the parameters evaluated, general condition, behavior, survival, body weights, water intake and hematological, and urinary parameters were not adversely affected at any dose. In the high-dose group, increased food intake associated with decreased food efficiency was reported, which were suggested to be attributed to the high levels of (inert) SAS material in the diet. Other slight changes were not considered to be of toxicological significance. Gross and microscopic pathological examinations did not reveal any abnormalities that could be attributed to the ingestion of the test article, SAS. The no-observed-effect level was reported to be the highest dose tested (8% in the diet or 4,000 mg/kg body weight/day) (ECETOC, 2006; GRN 554 – U.S. FDA, 2015).

Upon chronic administration of SAS to rats or mice for up to 24 months at concentrations of up to 5% in the diet, no changes in the survival rate, clinical observations, body weight, food consumption, organ weights, or blood chemistry ,and no gross or microscopic changes or neoplasms in any examined tissues were report ed. The authors of these studies concluded that administration of SAS to mice and rats did not result in any signs of carcinogenicity or other significant treatment-related adverse effects (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). No signs of reproductive or developmental toxicity were reported in studies conducted in rats, mice, hamsters, and rabbits administered SAS orally during gestation (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). SAS was not genotoxic or mutagenic when tested in a variety of *in vitro* and *in vivo* assays (ECETOC, 2006; GRN 554 – U.S. FDA, 2015).

6.5 Conclusions

Based on the above data and information presented herein, DouxMatok has concluded that SAS is GRAS, on the basis of scientific procedures, for use as a carrier in white sugar as described in Section 1.3. General recognition of the DouxMatok's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the intended use of SAS, who similarly concluded that the proposed uses of SAS are GRAS on the basis of scientific procedures.

SAS therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the *Code of Federal Regulations*.

Part 7. §170.255 LIST OF SUPPORTING DATA AND INFORMATION

Anguebes F, Pat L, Ali B, Guerrero A, Córdova AV, Abatal M, Garduza JP (2016). Application of multivariable analysis and FTIR-ATR spectroscopy to the prediction of properties in Campeche honey. J Anal Methods Chem 2016:Article ID 5427526 [14pp]. DOI:10.1155/2016/5427526.

CDC (2018a). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2015 [NHANES Home Page last reviewed: October 30, 2018].

CDC (2018b). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

Dazult Ltd. (2018). *DaDiet - The Dietary Intake Evaluation Tool [Software]*. (Version 17.04). Straffan, Ireland: Dazult Ltd. Available online: http://dadiet.daanalysis.com [Last accessed: Aug. 8, 2018].

ECETOC (2006). *Synthetic Amorphous Silica (CAS No. 7631-86-9).* (JACC No. 51). Brussels, Belgium: European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Available at: http://www.ecetoc.org/publication/jacc-report-51-synthetic-amorphous-silica/ [Sep. 2006].

EFSA (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies [NDA] related to the tolerable upper intake level of silicon (Question no: EFSA-Q-2003-018, adopted 28 April 2004 by the European Food Safety Authority). EFSA J 60:1-11. DOI:10.2903/j.efsa.2004.60. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/60.

EFSA (2009). Scientific Opinion of the Panel on Food Additives and Nutrient Sources Added to Food [ANS] on calcium silicate, silicon dioxide and silicic acid gel added for nutritional purposes to food supplements following a request from the European Commission (Question nos: EFSA-Q-2005-098, EFSA-Q-2005-099, EFSA-Q-2005-140, EFSA-Q-2006-220, adopted 5 June 2009 by the European Food Safety Authority). EFSA J 1132:1-24. DOI:10.2903/j.efsa.2009.1132. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/1132.htm.

EFSA (2018). Re-evaluation of silicon dioxide (E 551) as a food additive (EFSA Panel on Food Additives and Nutrient Sources Added to Food/ANS) (Question no: EFSA-Q-2011-00576, adopted 23 November 2017 by the European Food Safety Authority, last updated 21 March 2018). EFSA J 16(1):5088. DOI:10.2903/j.efsa.2018.5088. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5088.

EVM (2003). Risk assessment: silicon. In: *Safe Upper Levels for Vitamins and Minerals.* Report of the Expert Group on Vitamins and Minerals. London, UK: Food Standards Agency (FSA), Expert Group on Vitamins and Minerals (EVM). pp. 306-312. Available at: https://cot.food.gov.uk/committee/committee-on-toxicity/cotreports/cotjointreps/evmreport [May 2008].

FASEB (1979). *Evaluation of the Health Aspects of Certain Silicates as Food Ingredients*. (PB-301 402; SCOGS 61; Contract No. FDA 223-75-2004). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO) for Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Select Committee on GRAS Substances (SCOGS).

FCC (2018). Silicon dioxide. In: *Food Chemicals Codex, 11th edition*. Rockville (MD): United States Pharmacopeial Convention, pp. 1059-1060.

Fruijtier-Pölloth C (2012). The toxicological mode of action and the safety of synthetic amorphous silica-a nanostructured material. Toxicology 294(2/3):61-79. DOI:10.1016/j.tox.2012.02.001.

Gok S, Severcan M, Goormaghtigh E, Kandemir I., Severcan F (2015). Differentiation of Anatolian honey samples from different botanical origins by ATR-FTIR spectroscopy using multivariate analysis. Food Chem 170:234-240. DOI:10.1016/j.foodchem.2014.08.040.

Hu S, Hsieh YL (2014). Preparation of activated carbon and silica particles from rice straw. ACS Sustain Chem Eng 2(4):726-734. DOI:10.1021/sc5000539.

ICUMSA (2007a). *The Determination of Sugar Moisture by Loss on Drying - Official*. (ICUMSA Method GS $2_{/1/3/9}$-15). International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Berlin, Germany: Bartens The Sugar & Sweeteners Publisher. Information available at: https://www.bartens.com/product/2200/.

ICUMSA (2007b). *The Determination of the Particle Size Distribution of White Sugar and Plantation White Sugar by Sieving - Accepted*. (ICUMSA Method GS $2_{/9}$-37). International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Berlin, Germany: Bartens The Sugar & Sweeteners Publisher. Information available at: https://www.bartens.com/product/2208/.

ICUMSA (2011a). *The Braunschweig Method for the Polarisation of White Sugar by Polarimetry - Official (Reference) Method*. (ICUMSA Method GS $2_{/3}$-1). International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Berlin, Germany: Bartens The Sugar & Sweeteners Publisher. Information available at: https://www.bartens.com/product/2209/.

ICUMSA (2011b). *The Determination of Conductivity Ash in Refined Sugar Products and in Plantation White Sugar - Official*. (ICUMSA Method GS $2_{/3/9}$-17). International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Berlin, Germany: Bartens The Sugar & Sweeteners Publisher. Information available at: https://www.bartens.com/product/2212/.

IOM (2001). Arsenic, boron, nickel, silicon, and vanadium [Silicon]. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, Panel on Micronutrients). Washington (DC): National Academy Press (NAP), pp. 502-553. Available at: https://www.nap.edu/read/10026/chapter/15.

JECFA (1969). Silicon dioxide, amorphous; aluminium, calcium, magnesium, and sodium alumino silicates. In: *Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-Caking Agents and Certain Other Substances*. 13th Report of the Joint FAO/WHO Expert Committee on Food Additives, May 27, 1969-Jun. 4, 1969, Rome, Italy. (FAO Nutrition Meetings Report Series, No. 46A, WHO/Food Add/70.36. World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://www.inchem.org/documents/jecfa/jecmono/v46aje60.htm.

JECFA (1974a). 5.1. Anticaking agents. Others. Silicon dioxide and certain silicates (aluminium, calcium, sodium alumino silicate). In: *Toxicological Evaluation of Certain Food Additives with a Review of General Principles and of Specifications*. 17th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switzerland. (WHO Technical Report Series, No. 539; FAO Nutrition Meetings Report Series, No. 53). Geneva, Switzerland: Food and Agriculture Organization of the United Nations (FAO), Rome, Italy / World Health Organization (WHO), pp. 16, 35. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_539.pdf.

JECFA (1974b). Silicon dioxide and certain silicates. In: *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switzerland. (WHO Food Additives Series, No. 5). Geneva, Switzerland: Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je04.htm.

JECFA (1982). 3.1. Specific food additives, 3.1.6. Inorganic buffering agents: magnesium silicate. In: *Evaluation of Certain Food Additives and Contaminants*. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 19-28, 1982, Rome, Italy. (WHO Technical Report Series, No. 683). Geneva, Switzerland: World Health Organization (WHO), pp. 27, 42. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf.

JECFA (1986). Calcium aluminium silicate. In: *Evaluation of Certain Food Additives and Contaminants*. 29th Report of the Joint FAO/WHO Expert Committee on Food Additives [JECFA], Jun. 3-12, 1985. (WHO Technical Report Series, No. 733). World Health Organization (WHO), p. 24. Available at: http://apps.who.int/iris/bitstream/handle/10665/37285/WHO_TRS_733.pdf?sequence=1.

OECD SIDS (2005). Synthetic amorphous silica and silicates [7631-86-9, 112945-52-5, 112926-00-8: Silicon Dioxide; 1344-00-9: Silicic Acid, Aluminum Sodium Salt; 1344-95-2: Silicic Acid, Calcium Salt]. In: *SIDS [Screening Information DataSet] Initial Assessment Report for SIAM 19*. Oct. 19-22, 2004, Berlin, Germany. Paris, France: Organisation for Economic Co-Operation and Development (OECD) / United Nations Environmental Programme (UNEP) Publications. Available at: https://hpvchemicals.oecd.org/UI/handler.axd?id=81d3694a-a582-4fa8-a8f2-f771459b67ed [Printed, Revised & Last Updated: 17.06.2005].

Rovani S, Santos JJ, Corio P, Fungaro DA (2019). An alternative and simple method for the preparation of bare silica nanoparticles using sugarcane waste ash, an abundant and despised residue in the Brazilian industry. J Braz Chem Soc 30(7):1524-1533. DOI:10.21577/0103-5053.20190049.

SCF (1991). 1.3.2. Silicate(s) and silicone dioxide. In: *First Series of Food Additives of Various Technological Functions (Opinion Expressed on 18 May 1990).* (Food Science and Techniques, Reports of the Scientific Committee for Food, Twenty-fifth Series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_25.pdf.

Svečnjak L, Biliskov N, Bubalo D, Barišić D (2011). Application of infrared spectroscopy in honey analysis**.** Agric Conspec Sci 76(3):191-195.

Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H, Uesugi S (1988). Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. Acta Med Biol 36(1):27-56.

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 000321 [Synthetic amorphous silica, Boston (MA): Cabot Corporation].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=321 [Aug. 10, 2010 – FDA has no questions].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000554 [Synthetic amorphous silica, Parsippany (NJ): Evonik Corporation].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=554 [May 14, 2015 – FDA has no questions].

U.S. FDA (2019). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2019/title21.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.480	Silicon dioxide
182—Substances generally recognized as safe	182.1711	Silica aerogel

USDA (2018). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2015-2016.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: July 31, 2018].

APPENDIX A
GRAS Panel Consensus Statement

GRAS Panel Statement Concerning the Generally Recognized as Safe (GRAS) Status of Synthetic Amorphous Silica (SAS) as a Carrier in White Sugar

12 January 2021

INTRODUCTION

At the request of DouxMatok Inc. (DouxMatok), an Expert Panel (the "GRAS Panel") of independent scientists, qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, conducted a critical and comprehensive assessment of data and information pertinent to the safety of synthetic amorphous silica (SAS) to determine whether the intended uses of SAS as a flavor enhancer in white sugar, as described in Table A-1, would be Generally Recognized as Safe (GRAS) based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine); Professor Emeritus George C. Fahey, Jr. (University of Illinois); and Professor Emeritus Robert J. Nicolosi (University of Massachusetts Lowell).

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of publicly available scientific data and information compiled from the literature, which included an evaluation of available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended use of SAS. This dossier was prepared, in part, from a comprehensive search of the scientific literature performed at the request of DouxMatok through December 2020 and included information characterizing the identity and purity of the ingredient, the manufacture of the ingredient, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, and the safety of SAS.

Following its independent and collective critical evaluation, and on the basis of scientific procedures, the GRAS Panel unanimously concluded that SAS, meeting food-grade specifications and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS for use as a flavor enhancer in white sugar, as described in Table A-1. A summary of the information critically evaluated by the GRAS Panel is presented below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

DouxMatok intends to market food-grade SAS as a carrier in white sugar (sucrose). This is based on a proprietary technology (S1 technology) that has been developed by DouxMatok for flavor delivery, resulting in an increased perception of sweetness when consumed. Specifically, food-grade SAS, in the form of silica gel, is mixed mechanically with sucrose and then crystalized or dried. No chemical bonds are formed between sugar and SAS; instead, sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions. The sucrose crystals containing SAS produced using the S1 technology is referred to as 'DouxMatok Sugar' throughout this document. The final SAS content in the dry sugar crystals is 0.05 to 0.3%.

The product specifications for SAS meet the Food Chemicals Codex (FCC) 11th edition monograph for silicon dioxide (FCC, 2018). The average particle size of SAS used in the manufacture of DouxMatok Sugar ranges from 4.5 to 5.3 micrometers (μm) and, therefore, SAS would not fall under the definition of nanoparticles (*i.e.*, particles with a diameter of <100 nm). The GRAS Panel reviewed the results from 5 non-consecutive batches of SAS and concluded that the manufacturing process produces a consistent product that conforms to the established specifications.

The GRAS Panel, individually and collectively, critically evaluated the manufacturing process of SAS, which is consistent with cGMP. Synthetic amorphous precipitated silica and silica gels are manufactured using a wet process that involves an alkali metal silicate solution (also called "water glass") and acids, typically sulfuric acid. The process involves precipitation, filtration, washing, drying, milling, and granulation, followed by packing and shipping of the product. The size of the primary particles and the amount of aggregation and agglomeration are determined by the reaction conditions of pH, temperature, concentration, and amount of agitation. The manufacturer of SAS (GRACE) used in the production of DouxMatok Sugar stated that "*silica gels are generally manufactured under acidic conditions with primary particles in the range of 1 to 10 nanometers (nm) that upon drying quickly adhere to form aggregates ranging from 1 to 20 micrometers (μm)*".

The GRAS Panel also reviewed data based on attenuated total reflection in conjunction with Fourier transform infrared (ATR-FTIR) spectroscopy, demonstrating that once in water, sugar and SAS molecules are completely dissociated. These data further confirmed that no chemical bonds are formed between the sugar and SAS and, instead, the sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions.

REGULATORY STATUS OF SAS

There are 3 main types of silica (silicon dioxide; SiO_2) defined under Chemical Abstracts Service (CAS) No. 7631-86-9, which include (i) crystalline silica; (ii) amorphous (non-crystalline) silica, which is naturally-occurring or produced as a by-product in the form of fused silica or silica fume; and (iii) synthetic amorphous silica, or SAS. There exist various types of SAS, based on whether they are produced through a wet route (precipitated silica or silica gel, also known as hydrated silica or silica aerogel) or a thermal route (pyrogenic silica) (Fruijtier-Poelloth, 2012). Precipitated silica and silica gel are chemically identical (CAS No. 112926-00-8), but possess slightly different physicochemical properties (*e.g.*, pore size distribution; silica gel tends to have a narrower pore size distribution than precipitated silica) (EFSA, 2018). Colloidal silica (silica sol) is a stable dispersion of SAS in a liquid (generally water). The silica that is used in the preparation of DouxMatok Sugar is a silica gel type of SAS.

Silicon dioxide or SAS is a food additive that is permitted for direct addition to foods intended for human consumption in the United States (21 CFR §172.480 – U.S. FDA, 2019). SAS has GRAS status for use as an anticaking agent, defoaming agent, stabilizer, adsorbent, carrier, conditioning agent, chill proofing agent, filter aid, emulsifying agent, viscosity control agent, and anti-settling agent in a variety of food categories (GRAS Notice [GRN] 321 – U.S. FDA, 2010). SAS also has GRAS status for use as a multi-functional direct ingredient in a broad range of food categories at levels not to exceed 2% of the finished food, and also as an indirect ingredient in the manufacture of adhesives, coatings, defoaming agents, greases and lubricants, paper and paperboard, and polymers used as components of food-packaging material (GRN 554 – U.S. FDA, 2015). Currently, there are no regulatory provisions that would allow use of SAS as a carrier in white sugar (sucrose).

INTENDED USE AND ESTIMATED EXPOSURE

The purpose of embedding the SAS in the sugar is to improve the delivery of sucrose and increase its rate of dissolution. The process is based on a proprietary technology (S1 Technology) that has been developed by DouxMatok for flavor delivery by coating/loading food-grade silica particles with various nutritive and non-nutritive sweeteners to form a sweetener/carrier composition through non-covalent interactions (hydrogen and van der Waals). This would result in an increased perception of sweetness when consumed, thereby significantly reducing the amount of sugar needed to produce a desired level of sweetness in a food. The use of SAS in white sugar is consistent with the definition of a 'carrier', as defined by Codex as:

> "*A food additive used to dissolve, dilute, disperse or otherwise physically modify a food additive or nutrient without altering its function (and without exerting any technological effect itself) in order to facilitate its handling, application or use of the food additive or nutrient*" (Codex, 2018).

For the production of DouxMatok Sugar, food-grade silica gel is mixed mechanically with sucrose and then crystalized or dried. The final SAS content in the dry sugar crystals is 0.05 to 0.3%.

Consumption data and information pertaining to the intended food uses of SAS were used to estimate the *per capita* and consumer-only intakes of SAS for specific demographic groups and for the total United States (U.S.) population. There were a number of assumptions included in the assessment, which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain SAS at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that SAS will have 100% market penetration in the food category of white sugar.

On a consumer-only basis, the resulting mean and 90th percentile intakes of SAS by the total U.S. population from proposed food uses in the U.S. were estimated to be 45 mg/person/day (0.66 mg/kg body weight/day) and 102 mg/person/day (1.52 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of SAS were determined to be 53 mg/person/day (0.64 mg/kg body weight/day), and 138 mg/person/day (1.56 mg/kg body weight/day), respectively, as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 13 and 29 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes of 1.03 and 2.06 mg/kg body weight/day at the mean and 90th percentile intakes, respectively.

DATA PERTAINING TO SAFETY

The food-grade SAS intended for use as a carrier in DouxMatok Sugar is mixed mechanically with sucrose using a high shear mixer and then crystalized. DouxMatok demonstrated that throughout this process, no chemical bonds are formed between sugar and SAS molecules; instead, sugar and SAS molecules are held together *via* hydrogen and van der Waals interactions. Considering the absence of any chemical interactions between SAS and sucrose and given that SAS discussed herein is chemically representative of the SAS materials that were previously concluded to be GRAS (*i.e.*, GRNs 321 and 554), a discussion of publicly available data and information relevant to the safety of SAS is incorporated by reference to pivotal studies discussed in GRNs 321 and 554.

The use of SAS as a direct and indirect food ingredient has been concluded to be GRAS at levels up to 2% in the finished food product; these GRAS conclusions were notified to the U.S. Food and Drug Administration (FDA) and filed by the Agency under GRNs 321 and 554 without objection (U.S. FDA, 2010, 2015). Since the

SAS used in the production of DouxMatok Sugar is chemically and compositionally similar to the SAS materials that were previously concluded to be GRAS (*i.e*., GRNs 321 and 554), a discussion of publicly available data and information relevant to the safety of SAS is incorporated by reference to pivotal studies discussed in GRNs 321 and 554. The GRAS Panel noted that SAS used in the production of DouxMatok Sugar, similar to the materials in GRNs 321 and 554, does not fall under the definition of nanomaterials (particles with a diameter <100 nm). Therefore, this safety evaluation does not cover assessment of nano-silica.

The GRAS conclusions in GRNs 321 and 554 were on the basis of scientific procedures, supported by the publicly available data evaluated by various organizations, including the Select Committee on Generally Recognized as Safe Substances (SCOGS) (FASEB, 1979), the Organisation of Economic Co-operation and Development (OECD) Screening Information Data Sets program (OECD SIDS, 2005), and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (ECETOC, 2006). In addition, the safety of SAS as a food additive also has been the subject of evaluations by various authoritative bodies, including the European Food Safety Authority (EFSA), the Scientific Committee on Food (SCF), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1969, 1974a,b, 1982; SCF, 1991; EFSA, 2018).

To identify new data pertinent to the safety of SAS published since the GRAS status of SAS was last evaluated in 2014 (*i.e*., GRN 554), a comprehensive search of the published scientific literature was conducted for the period spanning from June 2014 through December 2020. The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Based on this updated search of the literature, DouxMatok is not aware of any newly published studies to suggest that SAS would be unsafe when used as a food ingredient.

A summary of the pertinent toxicological studies from prior GRAS notifications and newly identified studies or publicly available scientific evaluation relevant to the safety of SAS is provided in the sections that follow. Based on conclusions from previous expert panels on the GRAS status of SAS, corresponding “no questions” letters issued by the FDA for GRNs 321 and 554, the widespread history of use of SAS as a food additive globally, and conclusions from other authoritative and scientific bodies on the safety of SAS (*e.g*., SCOGS, OECD, JECFA, ECETOC, EFSA, and SCF), DouxMatok concluded that the current GRAS status of SAS as described in GRNs 321 and 554, can be extended to SAS and, therefore, SAS used in the production of DouxMatok Sugar, as described herein, is GRAS for use as a carrier, based on scientific procedures.

Metabolic Fate

TThe metabolic pathway of SAS has been evaluated as part of various safety assessments conducted by SCOGS, the OECD, and the ECETOC (FASEB, 1979; OECD SIDS, 2005; ECETOC, 2006) and GRNs 321 and 554 (U.S. FDA, 2010, 2015), and more recently as part of EFSA’s re-evaluation of silicon dioxide as a food additive (EFSA, 2018). The results of animal and human studies indicate that, following ingestion, SAS is not expected to undergo significant intestinal absorption and any small quantities of soluble SAS that is absorbed will be excreted unchanged in the urine. Accumulation in body tissues was reported to be limited with no indication of metabolism of SAS. The ECETOC (2006) report concluded that, “In contrast to crystalline silica, SAS is soluble in physiological media and soluble chemical species are formed which are eliminated via the urine without modification after intestinal resorption”.

Safety Evaluations of Synthetic Amorphous Silica (SAS)

Tolerable Upper Intake Level for Elemental Silicon

The nutritional role of elemental silicon has been considered by a number of authoritative bodies, including SCF of the European Commission, EFSA, the UK Expert Group on Vitamins and Minerals (EVM), and the Institute of Medicine of the United States National Academies of Sciences (IOM). Data relevant to the safety of silicon has been reviewed by these authoritative bodies during their derivation of a tolerable upper intake level (UL)[1] for elemental silicon.

Following a review of a 2-year carcinogenicity study, wherein SAS was orally administered to rats and mice at dietary levels of 0, 1.25, 2.5, and 5%, corresponding to theoretical doses of 0, 625, 1,250 or 2,500 mg/kg body weight/day in rats and 0, 1,875, 3,750, or 7,500 mg/kg body weight/day in mice, no evidence was found of silica-induced tumors or any other toxic effects (Takizawa *et al.*, 1988). The IOM concluded that typical levels of silicon intake from the diet would not pose any safety concerns for the general population (IOM, 2001). Using consumption data from dietary surveys conducted in the U.S., the IOM estimated that the median intake of elemental silicon for adult men and women ranged from approximately 14 to 21 mg/day (depending on the age category). Additionally, the IOM estimated the median intake of elemental silicon from dietary supplement sources in adults in the U.S. to be approximately 2 mg/day. IOM concluded that "*Due to lack of data indicating adverse effects of silicon, it is not possible to establish a UL*" (IOM, 2001). The EVM considered the feeding study in rats and mice conducted by Takizawa *et al.* (1988) to be the key study to derive a safe upper level (SUL) for daily supplemental intake of elemental silicon (EVM, 2003). Based on the findings of this study, the no-observed-adverse-effect level was concluded as 5%, or 50,000 ppm of supplemental silicon dioxide (theoretical doses of 2,500 mg/kg body weight/day in rats and 7,500 mg/kg body weight/day in mice), the highest doses tested. A dose of 2,500 mg silicon dioxide/kg body weight/day is equivalent to approximately 1,165 mg elemental silicon/kg body weight/day. After applying uncertainty factors of 10 for interspecies variations and 10 for interindividual variations, a safe upper-level intake of 12 mg elemental silicon/kg body weight/day was derived, which is equivalent to approximately 700 mg/day of elemental silicon for a 60-kg individual.

The Scientific Panel on Dietetic Products, Nutrition, and Allergies (NDA Panel) of EFSA acknowledged occurrence of kidney lesions reported in some animal studies following administration of silicates (EFSA, 2004). Due to the lack of suitable data to support a dose-response relationship for adverse effects following the intake of elemental silicon, a UL could not be derived. Nevertheless, the NDA Panel concluded that based on its long history of safe consumption, the estimated typical dietary intake level of silicon (*i.e.*, 20 to 50 mg/day, corresponding to 0.3 to 0.8 mg/kg body weight/day for a 60-kg individual) is unlikely to cause any adverse effects (EFSA, 2004). These silica (SAS) intakes correspond to silicon intakes of approximately 21 and 48 mg/day at the mean and 90th percentile, respectively. The intakes of elemental silicon from the proposed uses of silica (SAS) in DouxMatok Sugar are in the range of typical dietary intakes of silicon (*i.e.*, 20 to 50 mg/day), as reported by EFSA (2004).

[1] EFSA uses the term 'tolerable upper intake level' (UL) to refer to the maximum level of total chronic daily intake of a nutrient (from all sources) that is judged to be unlikely to pose a risk of adverse health effects to humans. The EVM uses the term 'safe upper level' (SUL) for this same concept (*i.e.*, the SUL represents an intake that can be consumed daily over a lifetime without significant risk to health on the basis of available evidence).

Safety Evaluation by the Select Committee on GRAS Substances (SCOGS)

The SCOGS conducted a comprehensive review of the use, exposure, and safety of silica and silicates in 1979 and stated that silicon dioxide and various silicates occur abundantly in practically all natural waters, animals, and plants, and thus are part of the normal human diet (FASEB, 1979). Silicon compounds that are used as direct food ingredients, with the exception of potassium and sodium silicates, are insoluble or very slightly soluble in water and appear to be biologically inert. The SCOGS also recognized that renal toxicity was reported in some animal studies following the ingestion of sodium silicate, magnesium trisilicate, and finely ground quartz. Since these effects had not been substantiated, SCOGS considers them to be species-specific. The SCOGS further noted that magnesium trisilicate, the predominant silicate added to foods in the U.S., is recognized as safe for use in large quantities as a component of antacid medicines in humans (FASEB, 1979).

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA first evaluated the safety of SAS (INS No. 551) and certain silicates (*i.e.*, aluminum, calcium, magnesium, and sodium alumina silicates) in 1969, and it was concluded that the use of these materials do not need to be limited, provided they are used in amounts consistent with Good Manufacturing Practice (JECFA, 1969). The safety of these compounds was re-evaluated by JECFA in 1973 as additional data became available, and a group acceptable daily intake (ADI) level of "not limited"[2] was established for silicon dioxide and aluminum, calcium, and sodium aluminosilicates (JECFA, 1974a,b). The Committee indicated that the available data supports the "biological inertness" of orally administered silica and silicates (JECFA, 1969, 1974). Even though additional data were not available by the meeting of 1982, the temporary qualifier for the ADI of "not limited/specified" was removed at that time, because the Committee decided to revise the specifications for magnesium silicate to exclude magnesium trisilicate (JECFA, 1982).

Scientific Committee on Food (SCF)

A safety assessment for the use of SAS (E 551) as a food additive was first conducted by the SCF in 1990 (SCF, 1991). Similar to the conclusions made by JECFA, the SCF established a group ADI level of "not specified" for SAS (E 551) and certain silicates (*i.e.*, sodium, potassium, calcium, and magnesium silicates[3]) when used as anticaking agents (SCF, 1991). Magnesium trisilicate was reported to have a history of safe use as an antacid in humans, without any adverse effects reported (SCF, 1991).

The European Food Safety Authority (EFSA)

In addition to its technological functions, SAS (E 551) may also serve nutritive purposes by acting as a source of elemental silicon. EFSA's Scientific Panel on Food Additives and Nutrient Sources added to Food published an opinion regarding the addition of calcium silicate, silicon dioxide, and silicic acid gel for nutritional purposes to food supplements (EFSA, 2009). The Panel concluded that the proposed use levels

[2] The ADI level of "not limited" established for silicon dioxide and certain silicates (aluminium, calcium, and sodium aluminosilicates) was subsequently reworded to "not specified" in 1985 (JECFA, 1986). The JECFA has replaced the term "not limited" with "not specified" to describe an ADI level for food substances of very low toxicity. In such cases, based on the available toxicological, biochemical, and clinical data, the total daily intake of the substance arising from its natural occurrence and/or its present use or uses in foods at the levels necessary to achieve the desired technological effect, will not represent a hazard to health. Any additive allocated an ADI of "not specified" must be used in accordance with GMP (*i.e.*, it is technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance).

[3] Silicates containing aluminum are required to comply with the Provisional Tolerable Weekly Intake (PTWI) established for aluminum of 7 mg/kg body weight (SCF, 1991).

of silicon dioxide in food supplements, which would provide up to 700 mg/day of elemental silicon, would not pose any safety concerns based on the available toxicity data. Upon re-evaluating the safety of silicon dioxide in 2017, for use as a food additive, the Panel concluded that, from the available database, there was no indication for toxicity of SAS (E 551) at the reported uses and use levels, but the Panel was unable to confirm the current ADI of "not specified". This was due to limitations reported in the toxicological database, more specifically, with respect to insufficient characterization of particle size distribution and, accordingly, recommended some modifications of the European Union specifications for E 551 (EFSA, 2018).

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)

Following a comprehensive review of the safety of SAS, the ECETOC Joint Assessment of Commodity Chemicals program reported that the results of several acute oral toxicity studies with various hydrophilic and hydrophobic types of SAS in rats and mice indicate a very low order of toxicity; no deaths and no signs of toxicity were reported at doses up to 5,000 mg SiO_2/kg body weight (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). A number of repeated-dose toxicity studies were conducted with SAS in rats confirming an absence of significant toxicity for SAS by oral route of exposure. The toxicity of a precipitated SAS product (trade name, SIPERNAT® 22) was evaluated in a 13-week study in Wistar rats, receiving the material at 0 (control), 0.5, 2, and 8% of the diet, corresponding to theoretical intakes of 0, 250, 1,000, and 4,000 mg/kg body weight/day. General condition, behavior, survival, body weights, water intake, and hematological and urinary parameters were not adversely affected at any dose. In the high-dose group, increased food intake associated with decreased food efficiency was reported, which was likely due to the high levels of (inert) SAS material in the diet. Other reported slight changes were not considered to be of toxicological significance. Gross and microscopic pathological examinations did not reveal any abnormalities that could be attributed to the ingestion of the test article, SAS. The no-observed-adverse-effect level (NOAEL) was reported to be the highest level tested (8% in the diet or 4,000 mg/kg body weight/day) (ECETOC, 2006; GRN 554 – U.S. FDA, 2015).

Chronic administration of SAS as a dietary admixture to rats or mice for up to 24 months at concentrations of up to 5% in the diet did not adversely affect survival, clinical observations, body weight, food consumption, organ weights, or blood chemistry, gross or microscopic changes, or neoplasms in any examined tissues. The authors of these studies concluded that administration of SAS to mice and rats did not result in any signs of carcinogenicity or other significant treatment-related adverse effects (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). No signs of reproductive or developmental toxicity were reported in studies conducted in rats, mice, hamsters, and rabbits administered SAS orally during gestation (ECETOC, 2006; GRN 554 – U.S. FDA, 2015). SAS was not genotoxic or mutagenic when tested in a variety of *in vitro* and *in vivo* assays (ECETOC, 2006; GRN 554 – U.S. FDA, 2015).

CONCLUSION

We, the members of the GRAS Panel, have, independently and collectively, critically evaluated the data and information summarized above, and unanimously conclude that the intended use as a carrier in white sugar (sucrose) of synthetic amorphous silica (SAS), manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting the food specifications presented in the dossier, is safe.

We further unanimously conclude that the intended use as a carrier in white sugar (sucrose) of synthetic amorphous silica (SAS), manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting the food specifications presented in the dossier, is Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

[redacted]

18 February 2021

Professor Emeritus Joseph F. Borzelleca
Virginia Commonwealth University School of Medicine

[redacted]

February 16, 2021

Professor Emeritus George C. Fahey, Jr.
University of Illinois

Date

[redacted]

14 February 2021

Professor Emeritus Robert J. Nicolosi
University of Massachusetts Lowell

Date

REFERENCES

ECETOC (2006). *Synthetic Amorphous Silica (CAS No. 7631-86-9).* (JACC No. 51). Brussels, Belgium: European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Available at: http://www.ecetoc.org/publication/jacc-report-51-synthetic-amorphous-silica/ [Sep. 2006].

EFSA (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies [NDA] related to the tolerable upper intake level of silicon (Question no: EFSA-Q-2003-018, adopted 28 April 2004 by the European Food Safety Authority). EFSA J 60:1-11. DOI:10.2903/j.efsa.2004.60. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/60.

EFSA (2009). Scientific Opinion of the Panel on Food Additives and Nutrient Sources Added to Food [ANS] on calcium silicate, silicon dioxide and silicic acid gel added for nutritional purposes to food supplements following a request from the European Commission (Question nos: EFSA-Q-2005-098, EFSA-Q-2005-099, EFSA-Q-2005-140, EFSA-Q-2006-220, adopted 5 June 2009 by the European Food Safety Authority). EFSA J 1132:1-24. DOI:10.2903/j.efsa.2009.1132. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/1132.htm.

EFSA (2018). Re-evaluation of silicon dioxide (E 551) as a food additive (EFSA Panel on Food Additives and Nutrient Sources Added to Food/ANS) (Question no: EFSA-Q-2011-00576, adopted 23 November 2017 by the European Food Safety Authority, last updated 21 March 2018). EFSA J 16(1):5088. DOI:10.2903/j.efsa.2018.5088. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5088.

EVM (2003). Risk assessment: silicon. In: *Safe Upper Levels for Vitamins and Minerals.* Report of the Expert Group on Vitamins and Minerals. London, UK: Food Standards Agency (FSA), Expert Group on Vitamins and Minerals (EVM). pp. 306-312. Available at: https://cot.food.gov.uk/committee/committee-on-toxicity/cotreports/cotjointreps/evmreport [May 2008].

FASEB (1979). *Evaluation of the Health Aspects of Certain Silicates as Food Ingredients*. (PB-301 402; SCOGS 61; Contract No. FDA 223-75-2004). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO) for Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Select Committee on GRAS Substances (SCOGS).

FCC (2018). Silicon dioxide. In: *Food Chemicals Codex, 11th edition*. Rockville (MD): United States Pharmacopeial Convention, pp. 1059-1060.

Fruijtier-Pölloth C (2012). The toxicological mode of action and the safety of synthetic amorphous silica-a nanostructured material. Toxicology 294(2/3):61-79. DOI:10.1016/j.tox.2012.02.001.

IOM (2001). Arsenic, boron, nickel, silicon, and vanadium [Silicon]. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, Panel on Micronutrients). Washington (DC): National Academy Press (NAP), pp. 502-553. Available at: https://www.nap.edu/read/10026/chapter/15.

JECFA (1969). Silicon dioxide, amorphous; aluminium, calcium, magnesium, and sodium alumino silicates. In: *Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-Caking Agents and Certain Other Substances*. 13th Report of the Joint FAO/WHO Expert Committee on Food Additives, May 27, 1969-Jun. 4, 1969, Rome, Italy. (FAO Nutrition Meetings Report Series, No. 46A, WHO/Food Add/70.36. World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://www.inchem.org/documents/jecfa/jecmono/v46aje60.htm.

JECFA (1974). Silicon dioxide and certain silicates. In: *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switzerland. (WHO Food Additives Series, No. 5). Geneva, Switzerland: Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je04.htm.

JECFA (1982). 3.1. Specific food additives, 3.1.6. Inorganic buffering agents: magnesium silicate. In: *Evaluation of Certain Food Additives and Contaminants*. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 19-28, 1982, Rome, Italy. (WHO Technical Report Series, No. 683). Geneva, Switzerland: World Health Organization (WHO), pp. 27, 42. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf.

JECFA (1986). Calcium aluminium silicate. In: *Evaluation of Certain Food Additives and Contaminants*. 29th Report of the Joint FAO/WHO Expert Committee on Food Additives [JECFA], Jun. 3-12, 1985. (WHO Technical Report Series, No. 733). World Health Organization (WHO), p. 24. Available at: http://apps.who.int/iris/bitstream/handle/10665/37285/WHO_TRS_733.pdf?sequence=1.

OECD SIDS (2005). Synthetic amorphous silica and silicates [7631-86-9, 112945-52-5, 112926-00-8: Silicon Dioxide; 1344-00-9: Silicic Acid, Aluminum Sodium Salt; 1344-95-2: Silicic Acid, Calcium Salt]. In: *SIDS [Screening Information DataSet] Initial Assessment Report for SIAM 19*. Oct. 19-22, 2004, Berlin, Germany. Paris, France: Organisation for Economic Co-Operation and Development (OECD) / United Nations Environmental Programme (UNEP) Publications. Available at: https://hpvchemicals.oecd.org/UI/handler.axd?id=81d3694a-a582-4fa8-a8f2-f771459b67ed [Printed, Revised & Last Updated: 17.06.2005].

SCF (1991). 1.3.2. Silicate(s) and silicone dioxide. In: *First Series of Food Additives of Various Technological Functions (Opinion Expressed on 18 May 1990).* (Food Science and Techniques, Reports of the Scientific Committee for Food, Twenty-fifth Series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_25.pdf.

Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H, Uesugi S (1988). Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. Acta Med Biol 36(1):27-56.

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 000321 [Synthetic amorphous silica, Boston (MA): Cabot Corporation].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=321 [Aug. 10, 2010].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000554 [Synthetic amorphous silica, Parsippany (NJ): Evonik Corporation].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=554 [May 14, 2015].

U.S. FDA (2019). U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2019/title21.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
170—Food additives	170.3	Definitions
172—Food additives permitted for direct addition to food for human consumption	172.480	Silicon dioxide

ANNEX A
Summary of the Individual Proposed Food Use and Use Level for Silica in the U.S.

Summary of the Individual Proposed Food Use and Use Level for Silica in the U.S.

Table A-1 Summary of the Individual Proposed Food-Use and Use Level for Silica in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food-Uses	Silica Use Levels (g/100 g)
Sugar, white, granulated	White Sugar	0.05 to 0.30

CFR = Code of Federal Regulations; U.S. = United States.

W. R. Grace & Co.-Conn.
7500 Grace Drive
Columbia, MD 21044

March 27, 2020

Re: GRAS Status of Silicon Dioxide in Grace Products

To Whom It May Concern:

Grace has conducted a review and determined that the silicon dioxide in its products which meet the Food Chemicals Codex (FCC) 10th edition monograph for silicon dioxide (INS 551) are Generally Recognized As Safe (GRAS) for the intended conditions of uses identified below when used in accordance with good manufacturing practices, used in an amount not in excess of that reasonably required to produce its intended effect, and used in an amount not to exceed 2 percent by weight of the food.

Conditions of Use
Anti-caking
Defoaming
Stabilizer
Absorbent
Carrier
Conditioning agent
Chillproofing agent
Filter aid
Emulsifying agent
Viscosity control agent
Anti-Settling agent

The above GRAS status also means that such silicon dioxide is authorized under 21 CFR 174.5(d)(1) for any use in food contact materials that does not have an intended technical effect on food so long as good manufacturing practice standards for food contact materials are maintained and it is used in an amount not in excess of that reasonably required to produce its intended effect.

If you have any questions, or need additional information please contact your local Grace representative or Brett Jurd of Grace's product stewardship group (contact details below).

This statement is valid without signature.

Brett Jurd
Product Stewardship Leader

W. R. Grace & Co.-Conn.
Columbia, Maryland 21044
410-531-4441 Office
410-531-4706 Fax
brett.jurd@grace.com

Disclaimers:
The above statement(s) are based on our current knowledge and experience and on legislation in effect on the date above. This compliance statement does not warrant against modifications of this product resulting from its processing or from the addition of other products, nor against any inadequate use and/or storage of this product or the materials and articles containing it. The present statement also does not warrant compliance with legislation changed after the date above.

5 October 2021

Katie Overbey, Ph.D., M.S
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Dear Dr. Overbey,

Re: FDA Questions on GRN 996

Question 1. On Pg. 40, you state that the notified ingredient is chemically representative of the ingredients that were subjects of GRNs 321 and 554. The manufacturing and identity described in GRN 996 is only for silica gel (CAS No. 112926-00-8). The ingredients in GRNs 321 and 554 are precipitated silica/silica gel (CAS No. 112926-00-8) and fumed or pyrogenic silica (CAS No. 112945-52-5). While they may be similar in chemical identity, their physical similarity is not apparent in your notice. Additionally, you mention that the ingredient adheres to form aggregates ranging from 1 to 20 micrometers upon drying.

Please provide physical characterization of the ingredient to demonstrate similarity to the ingredients notified in GRNs 321 and 554 and its capability to aggregate.

Please provide an accompanying narrative to conclude that, based on these comparisons, your article of commerce is expected to have the same physiological and toxicological profile as those that were previously submitted for our evaluation.

Further, if there are any differences, please provide a rationale as to why they do not impact your GRAS conclusion.

Response 1: The three types of SAS mentioned in GRNs 321, 554 and 996 are manufactured as follows:

1. Pyrogenic (fumed) silica is produced by hydrolysis of chlorosilanes in an oxygen-hydrogen gas flame. The following reaction occurs: $SiCl_4 + 2H_2O + O_2 \rightarrow SiO_2 + 4HCl$. In the reactor amorphous silica (5-50 nm) is fused into aggregates (100 nm) which then agglomerate (1-250 µm) in the cooling system.

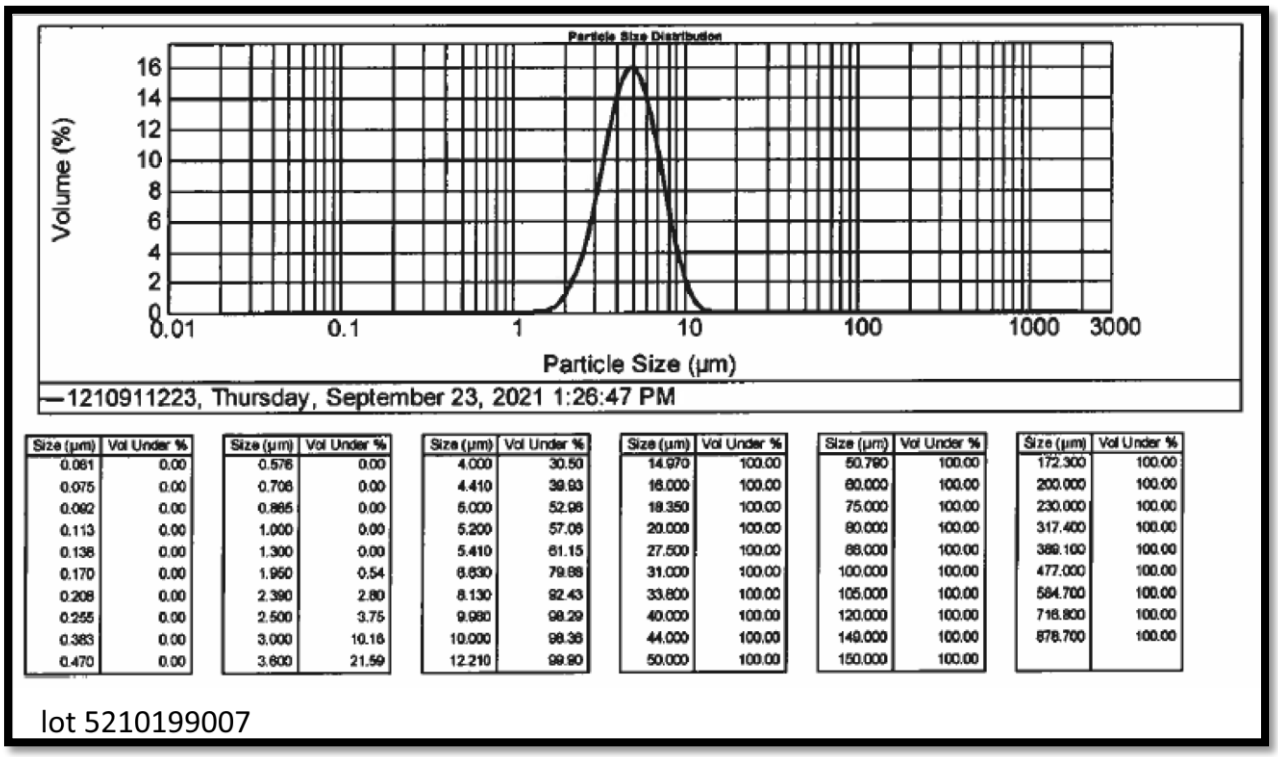
2. Precipitated silica is produced by reacting an alkali metal silicate solution (called "waterglass") with acid (sulfuric acid or hydrochloric acid), according to the following reaction: $nNa_2O{\cdot}xSiO_2 + nH_2SO_4 \rightarrow nNa_2SO_4 + xSiO_2 + nH_2O$. The precipitation formed is then filtered, washed, dried and milled.

3. Silica gel is produced similarly to precipitated silica by mixing alkali metal silicate solution (waterglass) with acid (sulfuric acid or hydrochloric acid) in a controlled manner to achieve a hydrosol. The reaction is the same as the precipitated silica: $nNa_2O{\cdot}xSiO_2 + nH_2SO_4 \rightarrow nNa_2SO_4 + xSiO_2 + nH_2O$.

The pyrogenic silica (CAS 112945-52-5) is strictly defined by its different production process. Precipitated silica (CAS 112926-00-8) and silica gel (CAS 112926-00-8) are chemically identical but show some minor

difference in physicochemical properties such as the distribution of pore size (*e.g.,* silica gel tends to have a narrower pore size distribution than precipitated silica). The functional physical parameters (pore size, particle size distribution, degree of agglomeration, surface area) are controlled by process conditions. Primary particles (5-50 nm) are clustered into aggregates *via* Si-O-Si covalent bonds which then attach to create agglomerates (1-50 µm). The agglomerates can be milled to the desired final particle size. The SAS used by DouxMatok in the production of the DouxMatok Sugar is purchased from GRACE, who have indicated that the SAS complies with the specifications for silicon dioxide established by the FCC (10^{th} edition), and are GRAS for use under specified conditions, including as a carrier, at levels up to 2% by weight of the food. The regulatory statement from the supplier of SAS is provided in Appendix 1. Furthermore, as highlighted in Section 2.2.1 of the GRAS Notice, the SAS used by DouxMatok in the production of the DouxMatok Sugar is manufactured similarly to precipitated silica (described in GRN 554), and the final product can be described as a silica gel, it is expected to have a similar physical profile as precipitated silica/silica gel. Furthermore, as highlighted in the GRAS Notice, food-grade SAS in the form of silica gel which conform to FCC specifications (12^{th} ed.), similar to the SAS described in GRNs 321 and 554 is used in the production of DouxMatok Sugar. The particle size of this food-grade silica gel confirms the silica gel is in an agglomerated form (see Figure 1 below). According to GRN 321 "*the smallest divisible, discrete entity of amorphous silica is an aggregate. An aggregate size for most solid SAS ranges from approximately 0.1 to 1 µm. Thus, solid powder forms of SAS do not exist as easily dispersible nanoparticles (i.e., particles with a diameter of < 100nm).*" For the production of the DouxMatok Sugar, the silica gel is mixed mechanically with sucrose using a mixer then the mixture is crystalized. For particle size analysis, the DouxMatok Sugar crystals are dissolved in water and the silica gel particles are analyzed. The results demonstrate that the particle size is not affected by the production process. Thus, considering that the SAS conforms to the FCC specifications (12^{th} ed.) of silicon dioxide, together with the particle size distribution data, it is concluded that the silica gel is chemically and physically similar to the SAS materials previously concluded to be GRAS (*i.e.,* GRNs 321 and 554; Cabot Corporation, 2010; U.S. FDA, 2010; Evonik Corporation, 2014; U.S. FDA, 2015) during the production of DouxMatok Sugar, and would therefore contain a similar toxicological profile (see Response 11).

Figure 1. Particle Size Distribution of SAS



Size (µm)	Vol Under %
0.081	0.00
0.075	0.00
0.092	0.00
0.113	0.00
0.138	0.00
0.170	0.00
0.208	0.00
0.255	0.00
0.383	0.00
0.470	0.00

Size (µm)	Vol Under %
0.576	0.00
0.706	0.00
0.865	0.00
1.000	0.00
1.300	0.00
1.950	0.54
2.390	2.80
2.500	3.75
3.000	10.16
3.600	21.59

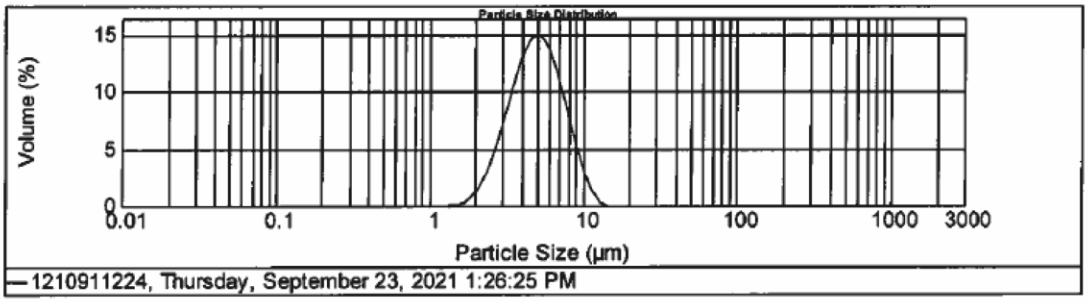
Size (µm)	Vol Under %
4.000	30.50
4.410	39.93
5.000	52.98
5.200	57.06
5.410	61.15
6.630	79.88
8.130	92.43
9.980	98.29
10.000	98.36
12.210	99.90

Size (µm)	Vol Under %
14.970	100.00
16.000	100.00
18.350	100.00
20.000	100.00
27.500	100.00
31.000	100.00
33.800	100.00
40.000	100.00
44.000	100.00
50.000	100.00

Size (µm)	Vol Under %
50.790	100.00
60.000	100.00
75.000	100.00
80.000	100.00
88.000	100.00
100.000	100.00
105.000	100.00
120.000	100.00
149.000	100.00
150.000	100.00

Size (µm)	Vol Under %
172.300	100.00
200.000	100.00
230.000	100.00
317.400	100.00
389.100	100.00
477.000	100.00
584.700	100.00
716.800	100.00
878.700	100.00

lot 5210199007



Size (µm)	Vol Under %
0.081	0.00
0.075	0.00
0.092	0.00
0.113	0.00
0.138	0.00
0.170	0.00
0.208	0.00
0.255	0.00
0.383	0.00
0.470	0.00

Size (µm)	Vol Under %
0.576	0.00
0.706	0.00
0.865	0.00
1.000	0.00
1.300	0.00
1.950	0.74
2.390	3.34
2.600	5.45
3.000	10.92
3.600	21.92

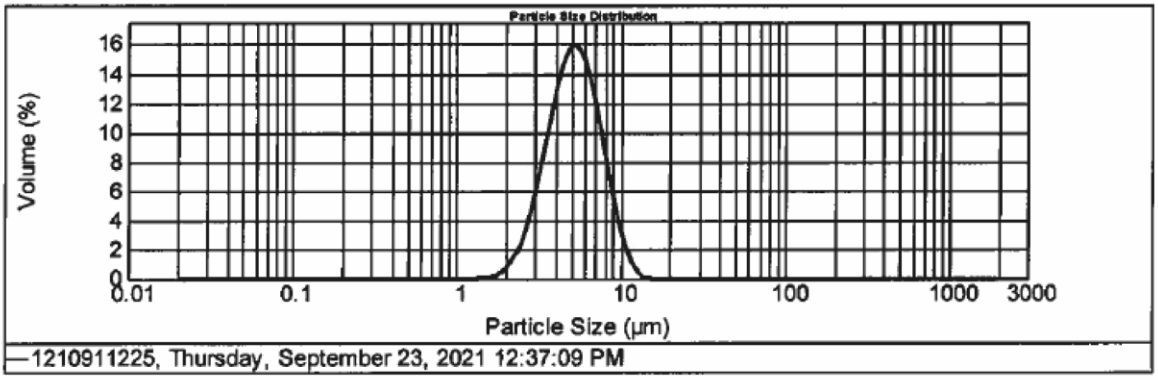
Size (µm)	Vol Under %
4.000	30.26
4.410	39.02
5.000	51.10
5.200	54.94
5.410	58.78
6.630	76.89
8.130	90.09
9.980	97.21
10.000	97.30
12.210	99.74

Size (µm)	Vol Under %
14.970	100.00
16.000	100.00
18.350	100.00
20.000	100.00
27.570	100.00
31.000	100.00
33.800	100.00
40.000	100.00
44.000	100.00
50.000	100.00

Size (µm)	Vol Under %
50.790	100.00
60.000	100.00
75.000	100.00
80.000	100.00
88.000	100.00
100.000	100.00
105.000	100.00
120.000	100.00
149.000	100.00
150.000	100.00

Size (µm)	Vol Under %
172.300	100.00
200.000	100.00
230.000	100.00
317.400	100.00
389.100	100.00
477.000	100.00
584.700	100.00
716.800	100.00
878.700	100.00

lot 5210199006



Size (µm)	Vol Under %
0.061	0.00
0.075	0.00
0.092	0.00
0.113	0.00
0.138	0.00
0.170	0.00
0.208	0.00
0.255	0.00
0.383	0.00
0.470	0.00

Size (µm)	Vol Under %
0.576	0.00
0.708	0.00
0.865	0.00
1.000	0.00
1.300	0.00
1.950	0.35
2.390	1.99
2.500	2.71
3.000	7.83
3.600	17.58

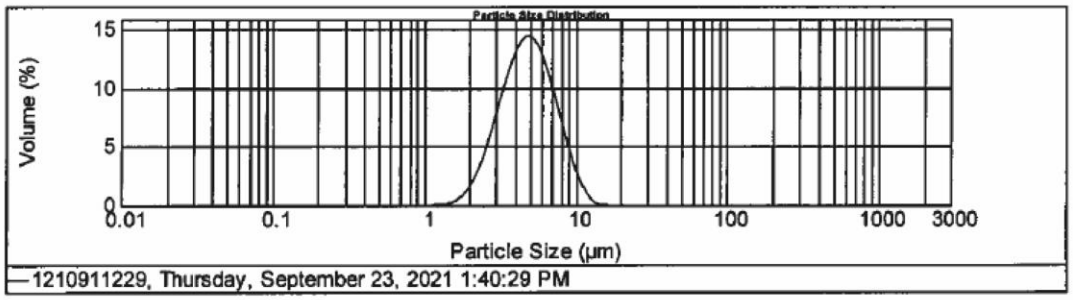
Size (µm)	Vol Under %
4.000	25.56
4.410	34.32
5.000	46.90
5.200	50.98
5.410	55.10
6.630	74.92
8.130	89.45
9.980	97.11
10.000	97.20
12.210	99.69

Size (µm)	Vol Under %
14.970	100.00
16.000	100.00
18.350	100.00
20.000	100.00
27.500	100.00
31.000	100.00
33.800	100.00
40.000	100.00
44.000	100.00
50.000	100.00

Size (µm)	Vol Under %
50.790	100.00
60.000	100.00
75.000	100.00
80.000	100.00
88.000	100.00
100.000	100.00
105.000	100.00
120.000	100.00
149.000	100.00
150.000	100.00

Size (µm)	Vol Under %
172.300	100.00
200.000	100.00
230.000	100.00
317.400	100.00
389.100	100.00
477.000	100.00
584.700	100.00
716.800	100.00
878.700	100.00

lot 5210199005



Size (µm)	Vol Under %
0.061	0.00
0.075	0.00
0.092	0.00
0.113	0.00
0.138	0.00
0.170	0.00
0.208	0.00
0.255	0.00
0.383	0.00
0.470	0.00

Size (µm)	Vol Under %
0.576	0.00
0.708	0.00
0.865	0.00
1.000	0.00
1.300	0.01
1.950	1.35
2.390	4.77
2.600	7.28
3.000	13.46
3.600	25.11

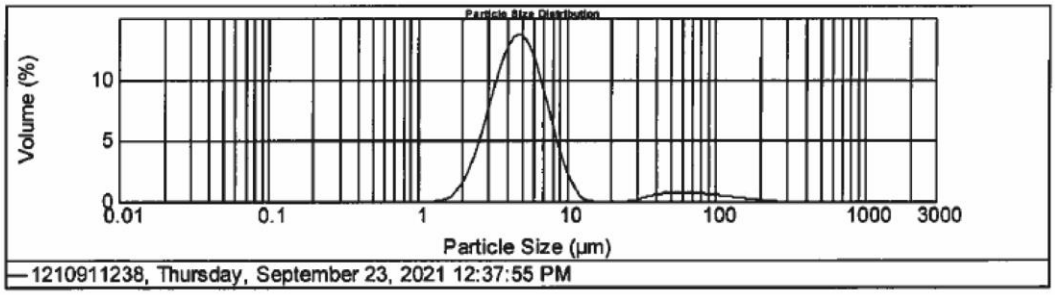
Size (µm)	Vol Under %
4.000	33.61
4.410	42.31
5.000	54.06
5.200	57.76
5.410	61.42
6.630	78.43
8.130	90.60
9.980	97.19
10.000	97.27
12.210	99.63

Size (µm)	Vol Under %
14.970	99.99
16.000	100.00
18.350	100.00
20.000	100.00
27.570	100.00
31.000	100.00
33.800	100.00
40.000	100.00
44.000	100.00
50.000	100.00

Size (µm)	Vol Under %
50.790	100.00
60.000	100.00
75.000	100.00
80.000	100.00
88.000	100.00
100.000	100.00
105.000	100.00
120.000	100.00
149.000	100.00
150.000	100.00

Size (µm)	Vol Under %
172.300	100.00
200.000	100.00
230.000	100.00
317.400	100.00
389.100	100.00
477.000	100.00
584.700	100.00
716.800	100.00
878.700	100.00

lot 5210199003



Size (µm)	Vol Under %
0.051	0.00
0.075	0.00
0.092	0.00
0.113	0.00
0.138	0.00
0.170	0.00
0.208	0.00
0.255	0.00
0.383	0.00
0.470	0.00

Size (µm)	Vol Under %
0.576	0.00
0.708	0.00
0.865	0.00
1.000	0.00
1.300	0.00
1.950	1.19
2.390	4.48
2.600	6.92
3.000	12.94
3.600	24.26

Size (µm)	Vol Under %
4.000	32.46
4.410	40.81
5.000	51.98
5.200	55.45
5.410	58.89
6.630	74.69
8.130	85.70
9.980	91.44
10.000	91.51
12.210	93.42

Size (µm)	Vol Under %
14.970	93.65
16.000	93.66
18.360	93.66
20.000	93.65
27.570	93.66
31.000	93.71
33.800	93.87
40.000	94.36
44.000	94.75
60.000	95.32

Size (µm)	Vol Under %
50.790	95.39
60.000	96.18
75.000	97.22
80.000	97.51
88.000	97.91
100.000	98.40
105.000	98.58
120.000	99.00
149.000	99.52
150.000	99.53

Size (µm)	Vol Under %
172.300	99.75
200.000	99.91
230.000	99.99
317.400	100.00
399.100	100.00
477.000	100.00
584.700	100.00
716.800	100.00
878.700	100.00

lot 5210199001

Question 2. On Pg. 6 of the notice, you state that "Silica gels are generally manufactured under acidic conditions with primary particles in the range of 1 to 10 nanometers (nm) that upon drying quickly adhere to form aggregates ranging from 1 to 20 micrometers (µm)." In Table 2.3-1 (p. 11), you specify the average particle size of SAS to be 4.5 to 5.3 µm. Please provide a particle size distribution of SAS for use as a carrier for sugar to demonstrate that it is made up of aggregates of SAS > 100 nm.

Response 2: The particle size distribution of SAS is provided in Figure 1. These results demonstrate that the particle size of the majority of the SAS used in DouxMatok Sugar falls within the range of 2.4 to 15 µm. These findings demonstrate that the SAS in DouxMatok Sugar has a particle size well above 100 nm, and would not be considered a nanoparticle.

Question 3. You have provided batch data for Loss on Ignition (LOI), Loss on Drying (LOD), and heavy metals as lead in Table 2.3-1 (p. 10). Please provide additional information listed below.

- Specify the lost organic contents from the LOI test.
- Based on the batch data for 5 non-consecutive lots of SAS, clarify how the sum of LOI, LOD, and Silica is greater than 100%.
- Clarify why the LOD is greater than the LOI for lot 5210181145.

Response 3. There are no volatile organic compounds (VOC) due to the fact that, according to the manufacturer (GRACE), during the manufacturing process no substance known or suspected to be a VOC has been used as a raw material or a processing aid. With respect to the sum of LOI, LOD, and silica, the content of silica is determined on the dry matter alone without the volatiles determined by LOD and LOI, and therefore should not sum up to 100% with LOD and LOI. Regarding the third point, according to the test methods, LOI is measured after the sample is pre-dried at 145°C for 4 hours. After the pre-drying stage, the sample is heated to 1,832°F (1,000°C), and held at the temperature for one hour. Therefore, it is possible for the LOD to be greater than the LOI.

Question 4. Provide a specification method that allows for a lower limit of detection of lead (≤ 4.5 ppm) and **Total heavy metals**. Specify the individual heavy metals for which you tested.

Response 4. The individual heavy metals tested in the DouxMatok SAS using USP 232/USP 233, including the limit of detection and results, are summarized in Table 1 below. The individual heavy metals analyzed are as follows: antimony, bismuth, chromium, cobalt, hafnium, cerium, copper, iridium, lithium, manganese, molybdenum, nickel, palladium, platinum, rhodium, selenium, silver, tin, thallium, uranium, vanadium, ruthenium, and osmium.

Table 1 Heavy Metals Analysis in 5 Production Batches of DouxMatok SAS

Heavy Metal (ppm)	Limit of Detection (ppm)	Manufacturing Lot No.				
		5210199007	5210199006	5210199005	5210199003	5210199001
Arsenic	0.0051	0.01	0.008	1.706	0.006	0.006
Cadmium	0.0005	0.004	0.003	0.169	0.004	0.004
Lead	0.0086	0.159	0.139	2.966	0.191	0.246
Mercury	0.0486	0.058	0.062	0.054	<0.03	<0.03

Question 5. On Pg. 6 of the notice, you state that "Silica gels are generally manufactured under acidic conditions with primary particles in the range of 1 to 10 nanometers (nm) that upon drying quickly adhere to form aggregates ranging from 1 to 20 micrometers (µm)." Please provide the pH value of the final product.

Response 5. The pH of 3 batches of the final product (measured after dispersion in water) is provided in Table 3.

Table 3 pH of 3 Batches of DouxMatok Sugar

Parameter	Lot No. 5210199004	Lot No. 5210199003	Lot No. 5210199001
pH	2.98	3	3.01

Question 6. Please provide a narrative, including food codes, mean, and 90th percentile values to address the cumulative dietary exposure to synthetic amorphous silica, to account for current and proposed uses.

Response 6. As noted in Section 1.3 of the GRAS Notice, DouxMatok intends to use SAS as a carrier in white sugar (sucrose). As discussed in GRN 554, the silicon content of the diets of male and female adults in the U.S. population were 40 mg/day and 19 mg/day, respectively, based on the Total Diet Study model (Pennington, 1991). These values were determined based on silicon content of grains, oats, barley, rice, *etc.* and were noted to be lower for animal-based diets compared to plant-based diets. Furthermore, GRN 554 discussed the average daily intakes of silica to be between 43 and 107 mg. For the purposes of a cumulative dietary exposure to SAS, 107 mg/day was used in the estimation. As discussed in Section 3.3 of the GRAS Notice, the highest dietary intake of SAS was 53 mg/day (mean) or 138 mg/day (90th percentile). In comparison to the highest background intake of 107 mg/day, the cumulative exposure in this population group is approximately 160 mg/day (mean) or 245 mg/day (90th percentile).

Question 7. Please update the FCC citation to the most current edition.

Response 7. The FCC citation has been updated to the 12th edition (FCC, 2021). The monograph is provided in Appendix 2.

Question 8. On pg. 19, you state:

"The ECETOC (2006) report concluded that, "in contrast to crystalline silica, SAS is soluble in physiological media and soluble chemical species are formed which are eliminated via the urine without modification after intestinal resorption."
However, EFSA (2018) responded to this conclusion by stating:
"The Panel noted that this was not supported by experimental data apart from limited human studies with few individuals where less than 0.5% of the orally applied SAS was excreted via urine, and urinary silicon was always within the range of normal physiological variation."
Please provide a discussion as to how EFSA's view impacts your safety conclusion.

Response 8. The conclusion by ECETOC (2006) appears to be related to pyrogenic SAS that was administered subcutaneously in rats as a suspension in water (Degussa, 1964; Klosterkötter, 1969 cited in ECETOC, 2006). Considering that the SAS ingredient, which is used for the production of DouxMatok Sugar is silica gel that will be orally administered as part of the DouxMatok Sugar, the conclusion made by ECTOC is not considered relevant to the safety conclusion in GRN 996 (U.S. FDA, 2021). The conclusion on the absorption, distribution, metabolism and excretion (ADME) of SAS in GRN 996 is aligned with findings from various ADME studies discussed in Sauer *et al*. (1959), JECFA (1974), SCF (1991), EFSA (2018), and ECETOC (2006). As discussed in the aforementioned studies and regulatory and scientific opinions, the available data on orally administered SAS reports the biological inertness of this substance and indicate that SAS is not absorbed or metabolized to a significant degree or systemically distributed in tissues and that most of the ingested SAS is excreted in the feces. These findings are also consistent with the safety narrative and the GRAS conclusion discussed in GRN 996.

Response 9. The citations of the mutagenicity/genotoxicity studies and preclinical safety studies as discussed in Sections 6.3.1 and 6.3.2 of the GRAS Notice, respectively, are provided in Tables 4 and 5 below. These studies were reviewed by authoritative and scientific bodies (*e.g.,* EFSA, OECD, JECFA, European Centre for Ecotoxicology and Toxicology of Chemicals) and the results discussed in Section IV.V of GRN 321 (pg. 4) and Section V.B.2 of GRN 554 (pg. 12) to support the GRAS status of SAS, which received "no questions" from FDA. Sections IV.V of GRN 321 (pg. 4) and V.B.2 of GRN 554 (pg. 12) are incorporated by reference to support the GRAS conclusions of SAS in DouxMatok Sugar. It is reiterated that DouxMatok purchases SAS from GRACE, who have indicated that their SAS conforms to FCC specifications (12th ed.) as described under GRN 321 and 554, and therefore has GRAS status in the U.S. The totality of evidence from the publicly available conclusions of authoritative and scientific bodies, as well as the previous GRAS conclusions of SAS, support the conclusion that SAS in DouxMatok Sugar is GRAS for use as a carrier in white sugar (sucrose). These GRAS conclusions are supported by a unanimous consensus rendered by an independent GRAS Panel, who similarly concluded that the proposed uses of SAS as a carrier in white sugar are GRAS on the basis of scientific procedures.

Table 4 Summary of Genotoxicity Studies for SAS – Silica Gel

Test	Test System/ Animal Species	Test Substance (Trade Name)	Concentration/Dose	Results	Reference
In Vitro					
Bacterial reverse mutation assay	Salmonella Typhimurium TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2	Silica gel (Silcron G-910)	Up to 10,000 µg/plate (±S9)	Negative	Mortelmans and Griffin (1981)
Bacterial reverse mutation assay	S. Typhimurium TA1530, G-46	Precipitated silica gel, crystalline-free (Syloid 244)	NR (-S9)	Negative	Litton Bionetics, Inc. (1974)

Table 4 Summary of Genotoxicity Studies for SAS – Silica Gel

Test	Test System/ Animal Species	Test Substance (Trade Name)	Concentration/Dose	Results	Reference
Chromosomal aberration assay	Human embryonic lung cells (Wi-38)	Precipitated silica gel, crystalline-free (Syloid 244)	1 to 1,000 µg/mL (-S9; 24 h)	Negative	Litton Bionetics, Inc. (1974)
Gene mutation assay	*Saccharomyces cerevisiae* D3	Precipitated silica gel, crystalline-free (Syloid 244)	NR (-S9)	Negative	Litton Bionetics, Inc. (1974)
Cytogenic assay	Human embryonic lung cells	Precipitated silica gel, crystalline-free (Syloid 244)	1 to 1,000 µg/mL (metabolic activation NR)	Negative	Cabot GmbH (1989)
Single-cell gel/Comet assay	Human embryonic lung cells	Silica gel (Spherisorb)	17.2 to 137.9 µg/mL (-S9; 3 h)	Positive (significant DNA migration ≥68.9 µg/mL)	Zhong *et al.* (1997)
Single-cell gel/Comet assay	Chinese hamster lung (V79) cells	Silica gel (Spherisorb)	17.2 to 137.9 µg/mL (-S9; 3 h)	Positive (significant DNA migration ≥68.9 µg/mL)	Zhong *et al.* (1997)
Micronucleus test	Chinese hamster lung (V79) cells	Silica gel (Spherisorb)	20 to 160 µg/mL (-S9; 24 h)	Positive (weak but significant induction of micronuclei)	Liu *et al.* (1996)
In Vivo					
Cytogenetic	Rat (Sprague-Dawley) M (5/group) Oral (gavage)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg Repeat-dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg 5 times per day	Negative	Litton Bionetics, Inc. (1974)
Dominant lethal assay	Rat (Sprague-Dawley) M, F (number of animals per group NR) Oral (gavage)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg Repeat-dose: 1.4, 14.0, 140, 500, and 5,000 mg/kg 5 times per day	Negative	Litton Bionetics, Inc. (1974)
Gene mutation assay (host-mediated)	Mouse (strain NR) and S. Typhimurium TA1530, G-46 (indicator)	Precipitated silica gel, crystalline-free (Syloid 244)	Single dose: 1.4 to 5,000 mg/kg Repeat-dose: 1.4 to 5,000 mg/kg; 5 times per day	Negative	Litton Bionetics, Inc. (1974)

-S9 = in the absence of metabolic activation; +S9 = in the presence of metabolic activation; F = female animals; h = hour(s); M = male animals; NR = not reported; SAS = synthetic amorphous silica.

Table 5 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Subchronic Studies						
Rat (CD-1) M, F (12/sex/group)	Oral (diet) 6 months	Precipitated silica gel, crystalline-free (Syloid 244)	M: 0, 2,170, or 7,950 F: 0, 2,420, or 8,980 (0, 3.2, or 10%)	Physical appearance Food consumption Growth Survival Hematology Clinical chemistry Blood chemistry Urinalysis Macroscopic examination Histology examination	• No treatment-related findings in any measured parameters • No effects on physical appearance, food consumption, growth, or survival • No clinical signs or effects on behavior and body weights observed • No effects on clinical chemistry observed • No histopathological changes observed in kidneys • NOAEL = 8,980 mg/kg bw/day	Grace (1975)
Rat (Wistar) 100 M	Oral (diet) 18 weeks	Sodium metasilicate	0, 100, 200, or 400 (0, 0.05, 0.1, or 0.2%)	NR	• No adverse effects reported	Najda *et al.* (1994)
Rat (Sprague Dawley) 5 M, 5 F	Oral (diet) 2 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	<u>Day 1 to 10:</u> 0, 5,800, or 16,500 (0, 5, or 10%) <u>Day 11 to 14:</u> 24,200 (20%)	Clinical symptoms Food consumption Water consumption Body weight gain Behavior	• No clinical symptoms observed • No effects on food or water consumption, body weight gain, or behavior observed	Grace (1974)
Rat (strain NR) 10 M/group	Oral (diet) 28 days	Micronized silica gel	0, 0.2, 1.0, or 2.5%	Mortality Abnormal gross autopsy Body weight gain	• No adverse effects observed • No mortality • No abnormal necropsy findings were observed • ↓ body weight gain [1.0, 2.5]	Keller (1958)

Table 5 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Reproductive and Developmental Toxicity						
Rat (Wistar) 20 to 25 F	Oral (gavage) GD 6 to 15	Precipitated silica gel, crystalline-free (Syloid 244)	0, 14, 63, 290, or 1,350	Clinical signs Survival Maternal body weight Abnormalities (soft and skeletal tissues)	F_0 • No effect on nidation or maternal/fetal survival • No maternal toxicity reported • NOAEL = 1,350 mg/kg bw/day F_1 • Soft and skeletal tissue abnormalities did not differ between treatment and sham-treated control groups • No developmental toxicity reported • NOAEL = 1,350 mg/kg bw/day	FDRL (1973)
Mouse (CD-1) 21 to 24 F	Oral (gavage) GD 6 to 15	Precipitated silica gel, crystalline-free (Syloid 244)	0, 13, 62, 290, or 1,340	Clinical signs Survival Number of abortions Live Litters Implantation sites Resorptions Dead and live fetuses Fetal weight Abnormalities (soft and skeletal tissues)	F_0 • No effect on nidation or maternal/fetal survival • ↓ maternal weight GD 15 and 17 [1,340] (no statistical evaluation conducted) • *"The relevance of this finding was questionable since the initial weight of dams in this group at GD 0 was 8% lower than in controls*" (EFSA, 2018) • NOAEL = 1,350 mg/kg bw/day F_1 • ↓ bw and skeletal retardation [1,340] (no statistical evaluation conducted) • Soft and skeletal tissue abnormalities did not differ • NOAEL = 1,350 mg/kg bw/day • "*The panel considered that in the absence of statistical evaluation the biological relevance of the reported changes cannot be evaluated*" (EFSA, 2018)	FDRL (1973)
Hamster (Syrian golden)	Oral (gavage) GD 6 to 10	Silica gel (Syloid 244)	0, 16, 74, 345, or 1,600	Clinical signs Maternal body weight	• No maternal or developmental toxicity observed	FDRL (1973)

Table 5 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Rabbit (Dutch-belted)	Oral (gavage) GD 6 to 18	Silica gel (Syloid 244)	0, 16, 74, 345, or 1,600	Clinical signs Maternal body weight Fetal weight Number of abortions Live litters Corpora lutea Implantation sites Early and late resorptions Dead and live fetuses Sex ratio External abnormalities Post-natal survival	• No differences in post-natal survival, abortions, and body weight gain during pregnancy • No dose-response effect observed in the ↑ of dead fetuses • ↓ average fetal weight [1,600] (no statistical evaluation conducted) • No developmental abnormalities observed • "*The Panel considered that in this study the documentation of data and the number of litters for fetopathological examination were not sufficient to reach a final conclusion*" (EFSA, 2018)	FDRL (1973)
Carcinogenicity						
Rat (Fischer 344) M, F (40/sex/group)	Oral (diet) 103 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	0, 625, 1,250, or 2,500 (0, 1.25, 2.5, or 5%)	Clinical sings Food consumption Survival Body weight Hematology Clinical chemistry Organ weight Tumor incidence	• No clinical signs observed • NS variations in survival rats (M, F) • No effects on body weight and food consumption • No treatment-related effects on hematology and clinical chemistry • ↓ liver weight from 12 to 24 months (F) [1,250; 2,500] (significance NR) • ↑ tumor incidence in testes and prepuce (M) (group and significance NR) • No pathologic or carcinogenic effects observed • NOAEL = 2,500 mg/kg bw/day	Takizawa *et al.* (1988)

Table 5 Summary of Preclinical Safety Studies for SAS – Silica Gel

Species (Strain), Sex, and Number of Animals	Route of Administration and Study Duration	Test Substance (Trade Name)	Dose in mg/kg bw/day (concentration)	Parameters Evaluated	Significant Findings[a,b]	Reference
Mouse (B6C3F1) M, F (40/sex/group)	Oral (diet) 93 weeks	Precipitated silica gel, crystalline-free (Syloid 244)	0, 1,875, 3,750, or 7,500 (0, 1.25, 2.5, or 5%)	Clinical signs Hematology Blood chemistry Urinalysis Gross examination Microscopic examination	• No clinical signs observed [up to 7,500] • ↑ food consumption (M, F) [3,750; 7,500], ↓ body weight gain between Weeks 15 to 50 (M) and Weeks 30 to 50 (F) [7,500] • "*No effects* of toxicological relevance on body weight (difference compared with control <10%) and food consumption" (EFSA, 2018) • NSD in survival rats or behavior observed • No dose-related effects in hematologic parameters • No sex- or dose-related effects in organ weights • No pathologic or carcinogenic effects observed • NOAEL = 7,500 mg/kg bw/day	Takizawa *et al.* (1988)

bw = body weight; F = female animals; F_0 = parental generation; F_1 = first filial generation; GD = Gestation Day; M = male animals; n = number of animals; NOAEL = no-observed-adverse-effect level; NR = not reported; NS = no significant; NSD = no significant difference; SAS = synthetic amorphous silica.

[a] Unless stated otherwise, all reported effects are statistically significantly different relative to control group(s).

[b] Information in [ ] indicates the dose at which effects were observed.

[c] The Panel considered that in the absence of statistical evaluation, the biological relevance of the reported changes cannot be evaluated.

Question 10. We note that in our literature search we found several more recently published, and potentially relevant, citations that were not listed in Part VII of your notice [e.g., (Boudard *et al.*, 2019; Hu *et al.*, 2019; Li *et al.*, 2018; Murugadoss *et al.*, 2020)].

- Please provide search terms used for your literature review and which citations were deemed relevant or not relevant in your GRAS conclusion.
- Please provide a narrative describing why these newly identified studies are not relevant to your GRAS conclusion; conversely, if any were identified as relevant, please discuss the studies in the context of the safety narrative you have presented and your GRAS conclusion.

Response 10: The search strategy is presented in Table 6 below. The scientific literature was searched for publications that have become available since the FDA's last review of the GRAS status of SAS in 2014 (*i.e.,* publications since GRN 554). The following databases were searched using ProQuest Dialog™: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. As discussed in Section 2.3 of the GRAS Notice, the average particle size of SAS ranges between 4.5 to 5.3 µm, and the particle size distribution of the SAS in DouxMatok Sugar ranges between 2.4 to 15 µm (see Response #2), indicating that the silica particles do not fall within the nanoparticle range (<100 nm). Therefore, publications on SAS nanoparticles were excluded from the literature search as

publications on the safety of SAS nanoparticles are not relevant to the safety of SAS in DouxMatok Sugar. We note that the publications identified in FDA's literature search relate to SAS nanoparticles which would have been excluded in our search findings. Similarly, the publications identified by the FDA relate to SAS nanoparticles (majority of SAS used were <50 nm; see Response 11), which are expected to have a different physical and chemical profile as SAS in DouxMatok Sugar, and would therefore not be relevant to the safety discussion of SAS in DouxMatok Sugar. Furthermore, it is noted that the SAS used by DouxMatok in the production of DouxMatok Sugar was previously concluded to be GRAS and is compositionally similar to SAS under GRN 321 and GRN 554, and FCC specifications for silicon dioxide (12th ed.).

Table 6 Search Strategy to Identify Publications Relevant to the Safety of DouxMatok's SAS

	Set No.	Search Terms
Substance Terms	S1	ti,ab(Silica or sodium silicate or silicic acid or silicon)
	S2	s1 not (mesoporous or lithium or fused or nanofiber or zeolite or nano* or pyrogenic or fumed or nanoparticle* or polymer* or crystal*)
Genotoxicity Terms	S3	s2 and ti,ab(genotox* or genetox*or mutagen* or mutat* or Ames or "dna repair" or "dna lesion*" or micronucle* or clastogen* or "DNA adduct*" or "comet assay*")
Preclinical Safety Terms	S4	s2 and ti,ab(animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet)
	S5	s4 and ti,ab(oral* or gavage or feed or feeding or diet or dietary or intub* or "drinking water" or intragastric or administ* or provid*)
	S6	s5 and ti,ab(toxic* or mortal* or lethal* or adverse* or safe* or risk* or hazard*)
	S7	s6 and ti,ab(acute* or subacute or "sub acute" or "single dose" or "short term" or subchronic* or "sub chronic*" or chronic* or "long term" or day or week or month or year)
	S8	s5 and ti,ab(LD50 or NOAEL or LOAEL or "no observed adverse effect*" or "low* observed adverse effect*" or NOEL or LOEL or "no observed effect level" or "low* observed effect level" or "maximum tolerated dose" or safety NEAR/2 assess* or risk NEAR/2 assess*)
	S9	s5 and ti,ab(carcino* or tumor* or tumour* or neoplas* or oncogen* or cancer*)
	S10	s5 and ti,ab(teratol* or teratogen* or reproduct* NEAR/5 toxic* or development* NEAR/5 toxic* or reproduct* NEAR/5 effect* or development* NEAR/5 effect* or fetus or foetus or fetal or foetal or prenatal* or postnatal* or perinatal* or litter or litters or "2 generation*" or "two generation*" or "multi generation*")
Combined Results	S11	s3 or s7 or s8 or s9 or s10
Clinical Safety Terms	S12	s2 and ti,ab(human or humans or subject or subjects or patient* or clinical* or volunteer* or men or women or male or female or "double blind*" or "single blind*" or "open label*" or "cross over" or crossover or cohort or randomiz* or randomis* or "placebo control*")
	S13	s12 and ti,ab(oral* or diet or dietary or ingest* or capsule or tablet or supplement* or consum* or provid* or administ*)
	S14	s13 and ti,ab(safe* or risk or "adverse effect*" or "adverse event*" or "adverse reaction*" or "maximum tolerated dose" or "permissible dose level" or "maximum dose level" or threshold or tolerability or tolera* or "side effect*")
Combined Results	S15	s11 or s14

Question 11. A recent publication was found that was likely published after you submitted your GRN (Brand *et al.*, 2021). Brand *et al.* state that "[t]he current review will help to progress research on the toxicity of SAS and the associated risk assessment." They further state:
"Altogether, this indicates that there are a lot of uncertainties and inconsistencies associated to the oral risk assessment of SAS. Yet, because different studies show effects at low external dose levels and at tissue concentrations that also occur in humans*, a human health risk as a result of oral exposure to SAS presently cannot be excluded.*" (emphasis added)

As all publicly available data and information should be evaluated as part of your GRAS conclusion, including those data that appear counter to your GRAS conclusion, please discuss how this publication would still support your GRAS conclusion.

Response 11. The publication by Brand *et al.* (2021) focused on the safety of SAS nanoparticles and evaluated a number of subchronic, repeated-dose oral toxicity studies that were published after 2014, the year of the group's last risk assessment (van Kesteren *et al.*, 2915). The studies that the authors evaluated were summarized in Table 1 of Brand *et al.* (2021). Upon closer evaluation, the particle size of the test articles of these publications were generally below 1 µm, with the majority of publications reporting particle sizes within the nanoparticle range (<100 nm). As discussed in Response 2, the SAS used in the production of DouxMatok Sugar is GRAS as an anticaking agent, defoaming agent, stabilizer, adsorbent, carrier, conditioning agent, chill proofing agent, filter aid, emulsifying agent, viscosity control agent, and anti-settling agent at levels not to exceed 2% of the finished food (a statement from the manufacturer GRACE is provide in Appendix 1). The final product itself has a particle size range between 2.4 and 15 µm; suggesting that SAS used in the manufacture of DouxMatox Sugar does not fall under the definition of a nanoparticle (<100 nm). Thus, SAS nanoparticles would not be representative of DouxMatok's SAS, which is compositionally similar to the SAS products that were concluded to be GRAS under GRN 321 and GRN 554, and meet FCC specifications (12th ed.) for silicon dioxide. Also, considering that the manufacturing process of DouxMatok Sugar would not change the particle size distribution of SAS from the SAS ingredient in GRNs 321 and 554, it is anticipated that the SAS ingredient in DouxMatok Sugar would share a similar safety profile to SAS in GRNs 321 and 554.

Sincerely,

David Tsivion,
CTO
DouxMatok Ltd

Encl. Appendix 1 – Regulatory Statement for SAS
Appendix 2 – FCC Monograph for Silicon Dioxide

REFERENCES

Brand W, van Kesteren PCE, Peters RJB, Oomen AG (2021). Issues currently complicating the risk assessment of synthetic amorphous silica (SAS) nanoparticles after oral exposure. Nanotoxicology 15(7):905-933. DOI:10.1080/17435390.2021.1931724.

Cabot Corporation (2010). *Generally Recognized as Safe Determination for Silicon Dioxide When Added Directly and/or Indirectly to Human Food: Silicon Dioxide GRAS Notification*. (GRN No. 321). Prepared by Washington (DC): Lewis & Harrison, LLC. Prepared for Boston (MA): Cabot Corporation. Submitted to: College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=321 [Aug. 10, 2010 - FDA response - no questions].

Cabot GmbH (1989) [unpublished]. *Cab-O-Sil - Salmonella/mammaliam-microsome Plate Incorporation Mutagenicity Assay (Ames Test)*. (Unpublished report, Microbiol. Assoc. No.: T9085.501). Cited In: OECD SIDS, 2005 [As: Cabot GmbH, 1989a].

Degussa (1964) [unpublished]. *Kurzgefasster Abschlussbericht über gewerbehygienisch-experimentelle Untersuchungen mit dem Kieselsäure-Füllstoff R972.* [Klosterkötter W]. Münster, Germany: Staatsinstitut für Staublungenforschung und Gewerbehygiene. Degussa, Hanau, Germany. Cited In: ECETOC, 20006 [As: Degussa, 1964a].

ECETOC (2006). *Synthetic Amorphous Silica (CAS No. 7631-86-9).* (JACC No. 51). Brussels, Belgium: European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Available at: http://www.ecetoc.org/publication/jacc-report-51-synthetic-amorphous-silica/ [Sep. 2006].

EFSA (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies [NDA] related to the tolerable upper intake level of silicon (Question no: EFSA-Q-2003-018, adopted 28 April 2004 by the European Food Safety Authority). EFSA J 60:1-11. DOI:10.2903/j.efsa.2004.60. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/60.

EFSA (2009). Scientific Opinion of the Panel on Food Additives and Nutrient Sources Added to Food [ANS] on calcium silicate, silicon dioxide and silicic acid gel added for nutritional purposes to food supplements following a request from the European Commission (Question nos: EFSA-Q-2005-098, EFSA-Q-2005-099, EFSA-Q-2005-140, EFSA-Q-2006-220, adopted 5 June 2009 by the European Food Safety Authority). EFSA J 1132:1-24. DOI:10.2903/j.efsa.2009.1132. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/1132.htm.

EFSA (2018). Re-evaluation of silicon dioxide (E 551) as a food additive (EFSA Panel on Food Additives and Nutrient Sources Added to Food/ANS) (Question no: EFSA-Q-2011-00576, adopted 23 November 2017 by the European Food Safety Authority, last updated 21 March 2018). EFSA J 16(1):5088. DOI:10.2903/j.efsa.2018.5088. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5088.

Evonik Corporation (2014). *GRAS Notification for Synthetic Amorphous Silica*. (GRN No. 554). Prepared by Parsippany (NJ): Evonik Corporation Submitted by Washington (DC): Keller and Heckman. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=554 [May 14, 2015 - FDA response - no questions].

FCC (2021). Silicon dioxide. In: *Food Chemicals Codex, Online edition*. Rockville (MD): United States Pharmacopeial Convention.

FDRL (1973). [Rats, Mice, Hamsters, Rabbits]. In: *Teratologic Evaluation of FDA 71-48 (Syloid; silica aerogel)*. (Final report 1573e, 1574e, 1575e, 1576e; PB-223 808; Report FDABF-GRAS-127). Prepared by East Orange (NJ): Food and Drug Research Laboratories (FDRL) for submission to Rockville (MD): U.S. Food and Drug Administration (U.S. FDA). Cited In: ECETOC, 2006 [As: US-FDA, 1973a,b,c,d].

Grace (1974) [unpublished]. *14-Tage-Vorversuch zu einer 6-Monate-Toxizitätsuntersuchung mit Syloid an Ratten bei oraler Applikation (Fütterungsversuch)*. (Inbifo Institut für biologische Forschung, Köln, unpublished report A 0386/1102). Worms, Germany: Grace. Cited In: ECETOC, 2006 [As: Grace, 1974c].

Grace (1975) [unpublished]. *6-Monate-Toxizitätsuntersuchung mit Syloid an Ratten bei oraler Applikation (Fütterungsversuch)*. (Inbifo Institut für biologische Forschung, Köln, unpublished report A0386/1103, Part 1 text, part 2 measuring values (table and diagrams), part 3 histologische Befunde [Tabellen]). Worms, Germany: Grace. Cited In: ECETOC, 2006 [As: Grace, 1975b].

JECFA (1969). Silicon dioxide, amorphous; aluminium, calcium, magnesium, and sodium alumino silicates. In: *Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-Caking Agents and Certain Other Substances*. 13th Report of the Joint FAO/WHO Expert Committee on Food Additives, May 27, 1969-Jun. 4, 1969, Rome, Italy. (FAO Nutrition Meetings Report Series, No. 46A, WHO/Food Add/70.36. World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://www.inchem.org/documents/jecfa/jecmono/v46aje60.htm.

JECFA (1974). Silicon dioxide and certain silicates. In: *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. Seventeenth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switzerland (WHO Food Additives Series No. 5; WHO Technical Report Series, No. 539; Nutrition Meetings Report Series, No. 53). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: World Health Organization (WHO). Available at: https://inchem.org/documents/jecfa/jecmono/v05je04.htm.

Keller JG (1958) [unpublished]. *Unpublished Report to W. R. Grace & Co.* Cited In: JECFA, 1969 [As: W.R. Grace in report and Keller in the reference list].

Klosterkötter W (1969) [unpublished]. *Gewerbehygienisches Gutachter über die hochdisperse Kieselsäure 'HDK V 15'*. Institut für Hygiene und Arbeitsmedizin. Burghausen, Germany: Wacker Chemie,. Cited In: ECETOC, 20006.

Litton Bionetics, Inc. (1974). *Mutagenic Evaluation of Compound FDA 71-48, Silica Aerogel*. (PB 245 467). Prepared by Kensington (MD): Litton Bionetics, Inc. for submission to Rockville (MD): U.S. Food and Drug Administration (U.S. FDA). Cited In: OECD SIDS, 2005; ECETOC, 2006 [As: US-FDA, 1974].

Liu X, Keane MJ, Zhong BZ, Ong TM, Wallace WE (1996). Micronucleus formation in V79 cells treated with respirable silica dispersed in medium and in simulated pulmonary surfactant. Mutat Res 361(2/3):89-94. DOI:10.1016/s0165-1161(96)90243-5. Cited In: ECETOC, 2006.

Mortelmans KE, Griffin AF (1981). *Microbial Mutagenesis Testing of Substances Compound Report: F76-037, Silica-Silcron G-910]*. (FDA/CFSAN-89/66; PB 89 187 066). Prepared by Menlo Park (CA): SRI International for Washington (DC): U.S. Food and Drug Administration (U.S. FDA). Cited In: ECETOC, 2006.

Najda J, Goss M, Gmínski J, Weglarz L, Siemianowicz K, Olszowy Z (1994). The antioxidant enzymes activity in the conditions of systemic hypersilicemia. Biol Trace Elem Res 42(1):63-70. DOI:10.1007/BF02990489. Cited In: EFSA, 2009.

OECD SIDS (2005). Synthetic amorphous silica and silicates [7631-86-9, 112945-52-5, 112926-00-8: Silicon Dioxide; 1344-00-9: Silicic Acid, Aluminum Sodium Salt; 1344-95-2: Silicic Acid, Calcium Salt]. In: *SIDS [Screening Information DataSet] Initial Assessment Report for SIAM 19*. Oct. 19-22, 2004, Berlin, Germany. Paris, France: Organisation for Economic Co-Operation and Development (OECD) / United Nations Environmental Programme (UNEP) Publications. Available at: https://hpvchemicals.oecd.org/UI/handler.axd?id=81d3694a-a582-4fa8-a8f2-f771459b67ed [Printed, Revised & Last Updated: 17.06.2005].

Pennington JA (1991). Silicon in foods and diets. Food Addit Contam 8(1):97-118. DOI:10.1080/02652039109373959.

Prival MJ, Simmon VF, Mortelmans KE (1991). Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutat Res 260(4):321-329. DOI:10.1016/0165-1218(91)90017-G. Cited In: ECETOC, 2006.

Sauer F, Laughland DH, Davidson WM (1959). Silica metabolism in guinea pigs. Can J Biochem Physiol 37(2):183-191. Cited In: ECETOC, 20006 [As: Sauer et al., 1959a].

SCF (1991). [Silicate]. In: *First Series of Food Additives of Various Technological* Functions (Opinion Expressed on 18 May 1990). (Food—Science and Techniques, Reports of the Scientific Committee for Food, Twenty-fifth Series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF). Available at: https://ec.europa.eu/food/system/files/2020-12/sci-com_scf_reports_25.pdf.

Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H, Uesugi S (1988). Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. Acta Med Biol 36(1):27-56. Cited In: ECETOC, 2006.

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 321 [Synthetic amorphous silica, Boston (MA): Cabot Corporation]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=321 [Aug. 10, 2010 - FDA response - no questions].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 554 [Synthetic amorphous silica, Parsippany (NJ): Evonik Corporation]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=554 [May 14, 2015 - FDA response - no questions].

U.S. FDA (2021). *Agency Response Letter GRAS Notice No. GRN 996 [Synthetic amorphous silica, Petah Tikva, DouxMatok Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=996 [Pending as of: Aug, 11, 2021 (Releasable data not yet avail.)].

van Kesteren PC, Cubadda F, Bouwmeester H, Van Eijkeren JC, Dekkers S, De Jong WH, et al. (2015). Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food. Nanotoxicology 9(4):442-452. DOI:10.3109/17435390.2014.940408.

Zhong BZ, Whong WZ, Ong TM (1997). Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay. Mutat Res 393(3):181-187. DOI:10.1016/s1383-5718(97)00094-6. Cited In: ECETOC, 2006.

Overbey, Katie

From: David Tsivion <david.tsivion@douxmatok.com>
Sent: Friday, February 4, 2022 10:44 AM
To: Overbey, Katie
Subject: [EXTERNAL] RE: GRN 996 - Additional FDA Questions

Dear Dr. Overbey,

I hope this finds you well. Please see the answers immediately after the questions:

1. Please state the sample sizes used for the total aerobic plate count, yeast and mold, gram negative bacilli, *Staphylococcus aureus*, and *Salmonella* specifications.

    - total aerobic plate count A1509 USP 61 – 10 g sample
    - yeast and mold – A1509 USP 61 – 10 g sample
    - gram negative bacilli – A1372 USP 62 – 10 g sample
    - *Staphylococcus aureus*- A1372 USP 62 – 10 g sample
    - *Salmonella* specifications – A1372 USP 62 – 10 g sample

2. Please confirm that the methods used for each of the microbiological specifications listed above is validated for the stated sample size.

    Yes, we confirm that the method used in each of the tests is validated for the stated sample size. All methods are validated for the 10 g sample size and all follow USP 61 and 62. See USP requirements:

    Amount of Sample required for USP <61> Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests

    10 Grams/mls/patches for USP 61 test

    Amount of Sample Required for USP <62> Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms

    10 Grams/mls/patches for USP 62 test

I am always available to answer any additional question.

Best regards,

David Tsivion, Ph.D
CTO

+972 52-8877117

DouxMatok | Incredo

From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Sent: Tuesday, February 1, 2022 4:47 PM
To: David Tsivion <david.tsivion@douxmatok.com>
Subject: GRN 996 - Additional FDA Questions

Dear Dr. Tsivion,

We had a few additional questions for GRN 996, please find them below.

Please format your response such that each answer immediately follows the stated question. Please ensure that your responses do not contain confidential business information and please do not submit a revised version of the GRAS notice.
We respectfully request a response to these questions within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options.

Please clarify the following information about the microbial specifications provided in Table 2.3-1:

1. Please state the sample sizes used for the total aerobic plate count, yeast and mold, gram negative bacilli, *Staphylococcus aureus*, and *Salmonella* specifications.
2. Please confirm that the methods used for each of the microbiological specifications listed above is validated for the stated sample size.

Best,
Katie

Katie Overbey, Ph.D., M.S (she/her/hers)
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240-402-7536
katie.overbey@fda.hhs.gov